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EFFECTS OF ELECTRICAL STIMULATION, HOT BONING AND FREEZING ON FUNCTIONAL CHARACTERISTICS OF EMULSIFIED MEAT PRODUCTS

by

BENJAMIN G. MEDINA

A THESIS

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled " Effects of Electrical Stimulation, Hot Boning and Freezing on Functional Characteristics of Meat Emulsified Products" submitted by Benjamin G. Medina in partial fulfilment of the requirements for the degree of Master of Science in Food Processing.

Dedicada a mis padres, Diego e Hilda,
y a todo lo que juntos compartiremos
mañana, Viviana.

ABSTRACT

The economical and technical advantages of hot processing and electrical stimulation, coupled with the greatly increased use of frozen meat in emulsified products in Canada necessitates the investigation of how these processes affect the meat protein functionality in sausage making.

The effects on protein functionality of electrical stimulation (ES), hot boning (HB), a combination of these methods (ESHB), and the conventional process were compared. The conventional or cold boned (CB) process was analyzed in both chilled and frozen meat (CBF). The functional properties of beef proteins were measured by different techniques such as water holding, water binding and emulsion capacities in the raw meat. Also, the measurement of water holding capacity and binding strength in the cooked emulsions were conducted.

At 72 h postmortem water holding capacities of 78.2, 57.2, 73.4, 70.9 and 75.4 % were found for CB, CBF, CBES, HB, and HBES meats, respectively. Emulsion capacities, expressed as ml oil/ mg total protein, of 0.92, 0.84, 0.97, 1.11, and 1.25 were determined for CB, CBF, CBES, HB, and HBES meats, respectively. At the same postmortem time the differently processed meats were emulsified and thermally treated. Binding strengths, in arbitrary units of 35.3, 29.9, 36.6, 30.1, and 31.8 were found for CB, CBF, CBES, HB, and HBES meats, respectively.

The main conclusions of this study are that all of the processes studied were less severe than freezing with respect to loss of protein functionality. Also, that a high value of emulsion capacity does not necessarily mean a good binding strength in the final product.

The results of this study reveal that neither ES or HB or combinations of these processes can substantially improve the functional properties of meats used in sausage making. As the use of frozen meat in the processed meat industry has been widely accepted, the incorporation of ES and HB in the packing plant may be possible considering that neither decreases the protein funtionality as much as freezing.

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1. INTRODUCTION

Meat is the postmortem condition of the three hundred or so anatomically distinct striated muscles of the animal body. The connective tissue in which the muscles fibres are deposited and the intermuscular fat that cannot be trimmed off without breaking up the muscle as a whole are also considered as constituents of meat.

The basic unit of striated muscular tissue is the fibre, which consists of formed protein elements, the myofibrils, bathed in a solution, the sarcoplasm, and a fine network of tubules, the sarcoplasmic reticulum. The muscle fibre is bounded by a very thin membrane called the sarcolemma, to which connective tissue is attached.

Living muscle is a highly specialized tissue that is capable of converting chemical energy into mechanical energy through its contractions. Muscles are positioned and attached to the skeleton in such a way that their contraction and relaxation lead to movement and locomotion. The ability to contract and relax, endowed on muscle via the thick and thin filaments of the myofibrils, is lost when muscle is converted to meat.

For many centuries people have used animal tissues for food without paying much attention either to their living functions, or to the changes that might take place in them before they are consumed. However, with the advent of the technological revolution, and centralized mass production techniques in the meat industry, a search continues for methods to control quality and uniformity in the end product. This has lead to investigations into the causes of variation in meat quality,

with emphasis towards quality improvement. A large number of the variations in meat quality are now known to be caused by changes that take place after slaughter. Animal tissues do not suddenly stop all living functions and become meat at the instant of slaughter. Instead, a number of physical and chemical changes take place over a period of several hours or even days.

New methods which are being considered in modern meat packing plants include electrical stimulation (ES) of carcasses immediately post slaughter, and removing meat from warm carcasses in the prerigor state (hot boning (HB)). Since these new practices may affect the quality of the meat, the major objective of this work was to investigate the manner in which meat protein functionality may be affected by these different processes. The effects of hot boning, electrical stimulation , and a combination of these (HBES) were compared to the standard (or conventional) process using both chilled and frozen meat.

The focus was on an attempt to determine how the meat proteins which are affected by these processes perform in the production of sausages. Functional properties of raw beef meat were measured by different techniques including water holding, water binding and emulsion capacities. Also, the water holding capacity and binding strength of cooked meat emulsions were measured.

1.1 Description of the processes

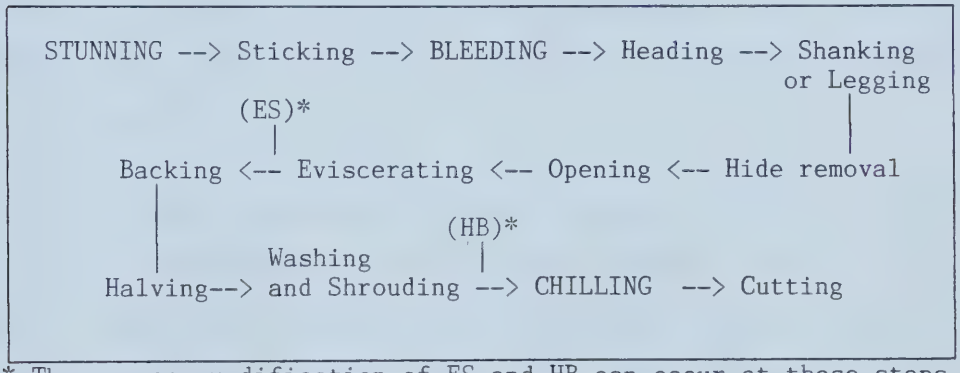
1.1.1 Conventional Process

The chemical and physical changes which take place in muscle after slaughter are associated with slaughtering conditions, ante and

postmortem treatment and storage conditions of carcasses as well as with physiological conditions and genetic traits (Kang et al., 1983). Generally speaking, the process of slaughter is similar in all cases. Most of the differences occur after the bleeding step.

The important steps that must be considered with respect to protein functionality, assuming normal microbiological conditions, are stunning, bleeding, carcass chilling (conditioning) and storage (Forrest et al., 1975, pg 166)(See Figure 1).

Figure 1 :Process of slaughter (after Romans and Ziegler, 1974).



* The process modification of ES and HB can occur at these steps.

1.1.1.1 Stunning and Bleeding

The properties and composition of muscle may be influenced by the type and effectiveness of the stunning process. It is desirable that whatever the method of stunning used the medulla oblongata in the brain should not be destroyed. This centre, which controls the lungs and heart, should continue to function for some time since the action of these organs helps to pump blood out of the carcass when the blood vessels in the neck are cut. Too much blood in the muscle causes an

unpleasant appearence and is an excellent medium for the growth of microorganisms.

1.1.1.2 Chilling or Conditioning

The temperature at which freshly slaughtered animal carcasses are stored can produce distinct changes in the rate of chemical reactions that occur in muscle tissue. Reactions catalyzed by enzymes in muscle are particulary temperature sensitive. Consequently the temperature after death should be lowered as quickly as possible to prevent protein denaturation and to inhibit the growth of microorganisms. Unfortunately, a too rapid reduction can cause cold shortening, and freezing meat prerigor can cause thaw rigor, which is very undesirable (Forrest et al., 1974, pg 168).

Thaw rigor is a severe type of rigor mortis which develops when muscle that has been frozen prerigor is thawed. This may cause a physical shortening of up to 80 percent of the original length of the unrestrained muscle fibers. Thaw rigor causes the release of large quantities of juices, which may amount to 30-40 percent of the muscle weight, and severe toughening (Bendall and Marsh, 1951). Muscle need not be frozen in order to get some of these undesirable attributes, as temperatures above 0 ^{o}C but below 15 - 16 ^{o}C, if attained prerigor, can cause a less severe type of contraction known as cold shortening.

When muscles go into rigor mortis in an extended condition, the filaments actin and myosin overlap and cross bond at fewer points, and the amount of actomyosin complex formed is small. Such meat is tender on cooking. On the other hand muscles which go into rigor mortis in a

contracted condition have considerable shortening since the actin and the myosin filaments interpenetrate extensively. There is much cross bonding and the meat is relatively tough on cooking(Lawrie, 1974, pg 138).

1.1.2 Freezing

When rigor mortis is finished and the natural heat of the muscle has been removed from the body, the meat can be frozen to prolong the useful storage life. One negative effect of this process is the exudation of fluid on thawing. This can lead to nutritive losses as proteins, peptides, amino acids, lactic acid, purines, vitamins of the B complex and various salts are among the many constituents of drip fluid (Lawrie, 1974, pg 203).

There are external and internal factors that influence the extent of drip in thawed meat. The external ones are related to geometry and size of the piece of meat (in particular, the ratio of cut surface to volume), orientation of cut surface with respect to muscle fiber axis, the prevalence of large blood vessels and the relative tendency for condensation or evaporation to occur in the thawing chamber (Lawrie, 1974, pg 203).

The internal factors are much more important and are related to the nature of the freezing process in the muscle tissue and to the water holding capacity of the muscle proteins. The rate at which the temperature of the meat falls is a most important consideration. When the freezing process is very fast (approximately 1 sec from 0 to -5 ^{o}C) water freezes between the actual filaments of myosin and actin in aggregates so small that they do not distort the structure even at the

level of observation possible with the electron microscope. Structural damage to the muscle increases as the duration of the freezing increases. (Lawrie, 1974, pg 203).

As extracellular ice formation proceeds at the usual slow rates, the remaining unfrozen extracellular fluid increases in ionic strength and drains water automatically from the super cooled interior of the muscle cell. This creates high ionic strength in the cells, which denatures some of the muscle proteins, and this factor (quite apart from the translocation of water) largely accounts for the loss of water holding capacity of the muscle proteins and for the failure of the fibres to reabsorb, on thawing, the water removed by freezing. Protein damage is a function of temperature and time of freezing. At -4 ^{o}C, for example, increasing damage is caused to both sarcoplasmic and myofibrillar proteins as time of storage increase (Awad et al., 1968).

1.1.3 Hot Processing

In many countries of the world, where refrigeration facilities are lacking, it is a common practice to cook meat in a prerigor state (hot boned). The technique of cutting beef, pork and lamb carcasses soon after slaughter (1 - 2 hours postmortem) and before conventional chilling, is described (even though not entirely accurately) as "Hot Boning" (HB) (Kastner, 1982). Just because a carcass is hot processed does not mean that the muscle is in a prerigor condition. In fact, some techniques of hot processing have been designed to avoid deboning before the onset of rigor mortis, whereas in others the attempt has been to capitalize on the functional properties of muscle in the prerigor state. The time to process the meat then must be related to the

chilling rate (Kastner, 1982).

1.1.3.1 Beef Hot Processing

Most of the early research on beef hot processing systems has relied on carcass and muscle or muscle system conditioning at elevated temperatures or the conventional aging of hot processed muscles to prevent or minimize any effects of cold shortening in beef roasts and steaks caused by prerigor excision. Hot processed beef to be used in ground meat has been found to be superior to cold processing in chemical, physical, cooking, and sensory properties (Berry and Stiffler, 1981; Cross and Tennent, 1981). These advantages have been attributed to the high pH value and high content of ATP of hot boned meat (Honikel and Hamm, 1978). In addition, Acton and Saffle (1969) claimed that postrigor beef has less emulsification capacity than prerigor beef when used in sausage making.

As seen in Figure 2, there are many systems for Hot Processing of beef, but all of them rely principally on the early postmortem muscle excision and elevated temperatures during conditioning (Kastner, 1982).

Extensive commercial application of hot boning under industrial conditions has not been realized, even though economic benefits and high quality products can result from the use of this practice. Included among the advantages are reduction in refrigeration and transportation, and less weight loss during cooling, which sum to a significant saving in processing time,labour, cost and energy (Henrickson, 1975).

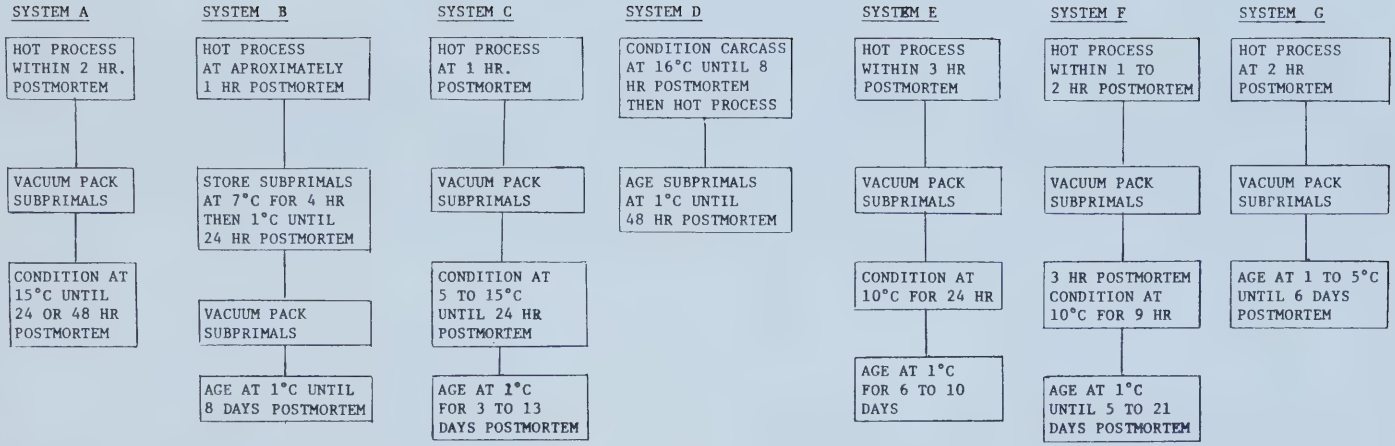

SYSTEM A
HOT PROCESS WITHIN 2 HR. POSTMORTEM
VACUUM PACK SUBPRIMALS
CONDITION AT 15°C UNTIL 24 OR 48 HR POSTMORTEM
SYSTEM B
HOT PROCESS AT APROXIMATELY 1 HR POSTMORTEM
STORE SUBPRIMALS AT 7°C FOR 4 HR THEN 1°C UNTIL 24 HR POSTMORTEM
VACUUM PACK SUBPRIMALS
AGE AT 1°C UNTIL 8 DAYS POSTMORTEM
SYSTEM C
HOT PROCESS AT 1 HR. POSTMORTEM
VACUUM PACK SUBPRIMALS
CONDITION AT 5 TO 15°C UNTIL 24 HR POSTMORTEM
AGE AT 1°C FOR 3 TO 13 DAYS POSTMORTEM
SYSTEM D
CONDITION CARCASS AT 16°C UNTIL 8 HR POSTMORTEM THEN HOT PROCESS
AGE SUBPRIMALS AT 1°C UNTIL 48 HR POSTMORTEM
SYSTEM E
HOT PROCESS WITHIN 3 HR POSTMORTEM
VACUUM PACK SUBPRIMALS
CONDITION AT 10°C FOR 24 HR
AGE AT 1°C FOR 6 TO 10 DAYS
SYSTEM F
HOT PROCESS WITHIN 1 TO 2 HR POSTMORTEM
VACUUM PACK SUBPRIMALS
3 HR POSTMORTEM CONDITION AT 10°C FOR 9 HR
AGE AT 1°C UNTIL 5 TO 21 DAYS POSTMORTEM
SYSTEM G
HOT PROCESS AT 2 HR POSTMORTEM
VACUUM PACK SUBPRIMALS
AGE AT 1 TO 5°C UNTIL 6 DAYS POSTMORTEM

Figure 2: Hot processing systems for beef. Adapted from Kastner (1982).

Despite all these merits, conventional hot processing techniques are not without some potential disadvantages. For example, hot processed meat is likely to be tough due to cold shortening if conditioned at 1 °C (Cross et al., 1980). Hot processed meat conditioned at a high temperature tends to be suceptible to microbial proliferation (Schmidt and Gilbert, 1970), however the study by Taylor et al. (1980) does not prove this contention. Inconvenience in cutting, maintaining the shape of the cuts and difficulties in synchronizing slaughtering, grading, hot boning and manufacturing time are some of the other drawbacks associated with conventional hot processing technique (Asghar and Henrickson, 1982). Many investigators have reported that most of the limitations arising from conventional hot processing can be solved by introducing an electrical stimulation unit in the carcass processing line (Asghar and Henrickson, 1982)

1.1.4 Electrical Stimulation

Although electrical stimulation (ES) has been adopted recently, its roots go back to the observation of Benjamin Franklin in 1749, on the beneficial effects of ES on the quality of turkey meat (Lopez and Herbert, 1975). In the early seventies, meat scientists in New Zealand initiated extensive studies on ES of lamb carcasses in an attempt to solve the problem of meat toughening caused by cold shortening. Their efforts were remarkably successful, and catalyzed much interest in the application of ES to other meat systems. Two systems of ES (low and high voltage) are now widely used. In the low voltage approach, the electricity usually is in the range of 20 to 90 volts, while the high voltage approach uses electricity in a range of 500 to 700 volts

(Savell, 1982).

There are three theories regarding the mechanism by which ES tenderizes meat. First, because the onset of rigor mortis is accelerated by ES, the muscle fibers do not shorten to the same extent that muscle fibers from unstimulated carcasses do when exposed to temperatures that cause cold shortening. Second, because of the rapid drop in pH caused by the hastened postmortem glycolysis while temperatures in the muscle are still high, conditions are favorable for the naturally occurring (lysosomal) enzymes, responsible for tenderization during the aging process, to degrade muscle proteins and cause tenderization. It has been demonstrated that the lysosomal enzymes in electrically stimulated muscle are released sooner and work at faster rates that lysosomal enzymes in unstimulated carcass muscles (Dutson et al., 1980). Third, light and electron micrographs from ES and unstimulated carcasses show that structural damage occurs in the muscle fibers of electrically stimulated samples (Savell et al., 1978).

It is possible that all three of these mechanisms are collectively responsible for the increase in tenderness related to ES. Whatever the situation, ES has been reported to be effective in improving the objective tenderness of steaks about 21 % (Savell, 1982). However, ES apparently does not increase the tenderness of all major muscles of the carcass, as has been shown by the fact that the chuck and blade steaks were not signicantly affected by ES, while that of other cuts of meat were improved by the process (Savell, 1982).

Only minor problems are associated with the water holding capacity of electrically stimulated beef even though the prerigor condition

associated with ES (a rapid decline in pH while the meat temperature is still high) is similar to the pale, soft, exudative condition in pork which does result in reduced water holding capacity (Savell, 1982).

As previously discussed, electrical stimulation of beef carcasses soon after slaughter can speed the onset of rigor mortis, thereby eliminating or minimizing toughness problems associated with hot processing, cold shortening and thaw rigor (Fig. 3).

1.1.5 Beef Electrical Stimulation and Hot Processing

Despite the fact that electrical stimulation decreases ATP content and lowers muscle pH, Cross and Tennent (1981) did not find any detrimental effects on the physical, chemical, cooking and sensory quality characteristics of ground beef patties made from such meat. Studies by Contreras et al. (1981) substantiate this finding, but patties made with electrically stimulated hot boned ground beef showed slightly higher cooking losses and more intense beef flavor than the control. The emulsion stability of sausages made from electrically stimulated carcass was claimed to be improved over those made from unstimulated meat (Whiting et al., 1981).

It is necessary to use high voltage stimulators if carcasses are to be hot processed, because very low voltage systems have proved less effective for use with hot processing (Bouton et al., 1980). Electrical stimulation indeed reduces the intensity of cold shortening, however the risk of some toughening will be present if the carcasses are chilled or frozen immediately after electrical stimulation (Asghar and Henrickson, 1982).

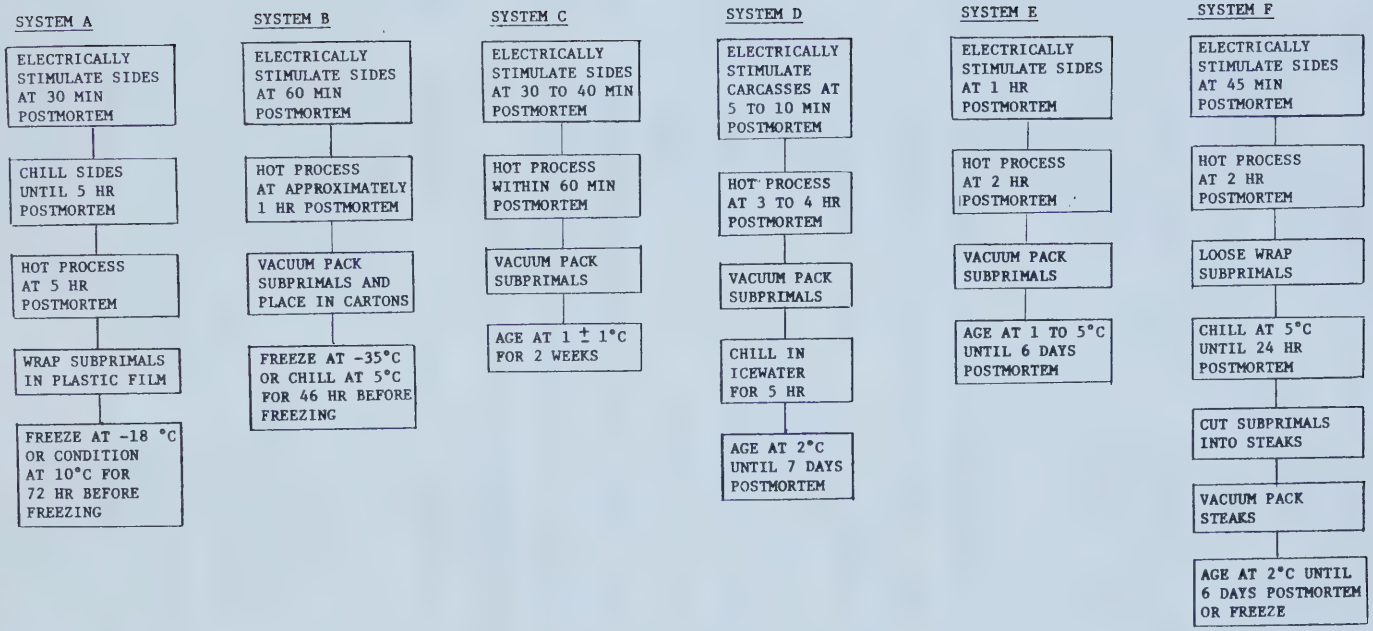

SYSTEM A
ELECTRICALLY STIMULATE SIDES AT 30 MIN POSTMORTEM
CHILL SIDES UNTIL 5 HR POSTMORTEM
HOT PROCESS AT 5 HR POSTMORTEM
WRAP SUBPRIMALS IN PLASTIC FILM
FREEZE AT -18 °C OR CONDITION AT 10°C FOR 72 HR BEFORE FREEZING
SYSTEM B
ELECTRICALLY STIMULATE SIDES AT 60 MIN POSTMORTEM
HOT PROCESS AT APPROXIMATELY 1 HR POSTMORTEM
VACUUM PACK SUBPRIMALS AND PLACE IN CARTONS
FREEZE AT -35°C OR CHILL AT 5°C FOR 46 HR BEFORE FREEZING
SYSTEM C
ELECTRICALLY STIMULATE SIDES AT 30 TO 40 MIN POSTMORTEM
HOT PROCESS WITHIN 60 MIN POSTMORTEM
VACUUM PACK SUBPRIMALS
AGE AT 1 ± 1°C FOR 2 WEEKS
SYSTEM D
ELECTRICALLY STIMULATE CARCASSES AT 5 TO 10 MIN POSTMORTEM
HOT PROCESS AT 3 TO 4 HR POSTMORTEM
VACUUM PACK SUBPRIMALS
CHILL IN ICEWATER FOR 5 HR
AGE AT 2°C UNTIL 7 DAYS POSTMORTEM
SYSTEM E
ELECTRICALLY STIMULATE SIDES AT 1 HR POSTMORTEM
HOT PROCESS AT 2 HR POSTMORTEM
VACUUM PACK SUBPRIMALS
AGE AT 1 TO 5°C UNTIL 6 DAYS POSTMORTEM
SYSTEM F
ELECTRICALLY STIMULATE SIDES AT 45 MIN POSTMORTEM
HOT PROCESS AT 2 HR POSTMORTEM
LOOSE WRAP SUBPRIMALS
CHILL AT 5°C UNTIL 24 HR POSTMORTEM
CUT SUBPRIMALS INTO STEAKS
VACUUM PACK STEAKS
AGE AT 2°C UNTIL 6 DAYS POSTMORTEM OR FREEZE

Figure 3: Electrical stimulation and hot processing systems for beef. Adapted from Kastner (1982).

If muscle pH has dropped below pH 6.0 before chilling, cold shortening does not occur to any significant degree. Therefore, pH 6.0 can be used as a standard to determine when to expose the carcasses to fast cooling temperatures. The pH of the muscle should be allowed to reach 5.7 before transfering the meat to the freezer, if blast freezing of hot processed meat is desired. This may take 3 to 4 hours if the carcasses are held at 18 °C after electrical stimulation (Bendall, 1973).

Several hot boning systems incorporate electrical stimulation to assure the tenderness of steaks and roasts (Fig. 3), although other parts of the carcass are used in the production of emulsified products. If the objective is to use prerigor meat in sausage production, electrical stimulation will decrease the available postmortem time before the muscle must be preblended in order to capitalize on the prerigor properties of muscle (Hamm, 1978). While several studies have pointed to the utility of prerigor muscle in sausages (Kastner, 1982), other studies on the influence of ES on the functional properties of prerigor muscle point to the need for additional research in this area (Terrel et al., 1982).

However, good quality products are not only dependent upon raw materials, they also depend on the processing techniques used. In addition, knowledge concerning to the relationships between the many active ingredients present in meat emulsion helps to obtain better quality. For these reasons an explanation of the main ingredients and the systems used to asses their efficiency will be presented in the following section.

1.2 The Major Components in Meat Emulsions

To the sausage processor, lean and protein are synonymous; lean meats contribute greatly to emulsion stability and to the physical properties of the finished product. The total protein content of commercial products is generally 11 to 12%, with proteins as a group representing the most important chemical component of this system. During the preparation of meat emulsions, meat proteins serve two primary functions: to emulsify fat and to bind water. If either of these is not accomplished properly, emulsions will be unstable and subject to breakdown during cooking (Randall, 1978).

1.2.1 Proteins

The proteins of major importance in sausage making can be grouped under myofibrillar and connective tissue proteins (collagen). The myofibrillar proteins (about 60% of the total muscle protein) are readily soluble in concentrated salt solutions. They are grouped as myosin, actin, actomyosin, the tropomyosins, the troponins and the actinins. Myosin is the most abundant of these proteins; during the onset of rigor mortis myosin and actin combine to form actomyosin. It is in this state that myosin and actin are generally used in meat emulsion systems. Myosin is the most surface active protein of meat and apparently has the ability to migrate to and locate at interfaces and unfold to form a cohesive matrix around fat droplets. During subsequent heating , the membrane material is set thermally and the simultaneous gelation of the protein matrix helps to stabilize the emulsion (Kinsella, 1982).

Collagen proteins are able to absorb a large amount of water when cold, but this water is released on heating during cooking process and if too much collagen is present in the formula, gelatin will be formed. During the emulsification process, the water absorption by collagen in formulations with a high collagen content must be a factor in the emulsification effectiveness of the myosin proteins (Rust, 1976). Collagen is easily converted to soluble gelatin by prolonged treatment at temperatures above the thermal shrinkage levels (70 °C); when heated to 60 - 70 °C, collagen fibres shrink to about one-third of their original length. These two physical properties of collagen limit its usefulness in emulsion-type products and for these reasons no more than 25% of the total protein should be present as collagen (Randall, 1978). If too much collagen is present the sausages twist (called the "Cork Screw Effect").

It is generally assumed that any technique that increases the quantity of salt soluble protein in skeletal muscle emulsions will be beneficial in improving emulsions. In a commercial system, this is accomplished by reducing the particle size of the meat, thus facilitating the liberation of the proteins into the water phase; and in the presence of salt they are even more readily solubilized. An improvement in solubility is obtained by increasing the salt concentration by raising the pH, or by the use of prerigor meats.

1.2.2 Fats

Fat is an essential component of processed meat products, and makes a large contribution to their palatability, because its presence directly affects the tenderness and juiciness of sausages. Also it is

considered as the dispersed phase in meat emulsions (Forrest et al., 1975, pg 210).

The fat contained in fatty tissue is primarily composed of triglycerides, with only palmitic, stearic and oleic acids present in substantial quantities. Small amounts of other lipids such as phospholipids and sterols are also present (Randall, 1978).

The fatty tissues derived from the different animal species have quite different melting points. Mutton fat is the hardest followed closely by beef and then by the softer pork fat. Using differential thermal analyses, it has been shown (Townsed et al., 1968) that there are two principle melting ranges for animal fats: 8.0-14.0 ^{o}C and 18.0-30.0 ^{o}C, beef and mutton having significantly more of the higher melting fraction. Emulsion stability during cooking has been shown to be related to this higher melting range. This coincides with what is common knowledge in commercial practice; that is, beef and mutton fat are more difficult to emulsify than pork fat (Randall, 1978).

On the other hand, there is an inverse relationship between temperature and emulsifying capacity of protein and, in addition, salt-soluble protein is more readily extracted at lower temperatures. So, there is a condition at which higher temperatures are preferred when considering the fat, but lower temperatures are required for most effective utilization of the protein. It is well established that small fat globules are characteristic of a stable emulsion and it has been suggested that fat globule size should be used as the end point for the fat chopping and emulsification steps in sausage making (Sulzbacher, 1973).

1.2.3 Water

Water composes 50 to 60% of the final weight, and is the largest component in meat emulsified products. Its presence originates from two sources, the water naturally present in the muscle and ice or water added depending upon the initial temperature of emulsion mix at time of addition. Besides its effect over the textural parameters water is also important for its chillling, lubricating and solubilizing properties in the meat mass during comminution (Kramlich et al., 1973).

In water, hydrogen atoms can become linked to other molecules through the hydrogen bond. This bonding plays an important role in maintaining protein structure and has been identified as playing a key role in the proteins' functionality in meat emulsion formation, stability and structure properties (Randall, 1978).

1.3 Description of Functional Properties

1.3.1 Use of Model Systems

Manufacture of meat emulsions was empirical until about two decades ago. The factors affecting the processing and quality of products were usually studied under practical conditions. However, this approach had various disadvantages, such as the size of the sample and the multiplicity of elements involved that are difficult to control and detect at the plant site. In-plant testing gives results of very limited applicability. The above limitations create the need to use model systems to investigate the role of the numerous ingredients/components and their properties. The most widely measured property in a model system is emulsion capacity. Emulsion capacity (EC) is measured to solve

fat retention/binding problems in emulsified meat products. Using the emulsifying capacity of the salt soluble proteins, binding values (a measure of the ability of the meat emulsions to hold together after thermal processing) of various meat ingredients have been determined using some model systems (Borton, 1968; Porteus, 1979). Such values have been used sucessfully as the basis of Least Cost Formulation (LCF) Programs. This fact either proves the value of these model systems and their correlation to final quality and other characteristics of formulated products or indicates that most products whose ingredients are calculated by LCF are so vastly overformulated with respect to certain parameters (e.g. collagen) that nothing could possibly go wrong during the processing and subsequent consumption (Randall, 1978).

Since these model systems vary considerably from the conditions found in commercial practice, the findings from model systems should be applied using great caution. In the work of Swift et al.(1961), emulsion breakdown under experimental conditions occurred at a protein to fat ratio of 1:1600, whereas in actual manufacturing practice a ratio of 1:4 will never be exceeded in order to emulsify/bind the fat properly. This situation emphasizes the need for improved standarized laboratory methods to evaluate the properties of proteins and determine the effect of different treatments on the functionality of these components.

1.3.2 Functionality of Proteins

Functional properties of proteins are defined as chemical and physical properties which affect the behavior of proteins in food systems during processing, storage, preparation and consumption. These characteristics (Table 1) influence and sometimes define the

Table 1: Typical functional properties performed by functional proteins in food (Kinsella, 1982).

Functional Prop.	Mode of action	Food system
Water absorption and binding	Hydrogen bonding of water; Entrapment of of water (no drip)	Meat, Sausages, Breads, Cakes
Gelation	Protein matrix formation and setting	Meats, Curds, Cheese
Cohesion/adhesion	Protein acts as adhesive material	Meat, Sausages, Baked goods, Pasta products
Elasticity	Hydrophobic bonding in gluten; Disulphide links in gels	Meats, Bakery
Emulsification	Formation and stabilization of fat emulsions	Sausages, Bologna, Soups, Cakes
Fat absorption	Binding of free fat	Meats,Sausages, Doughnuts
Flavor binding	Absorption, entrapment, release	Simulated meats, Bakery, etc.

quality and organoleptic attributes of food (Kinsella, 1982).

The type of functional property required in a protein or in a protein mix varies with the particular food system in study. Thus, in a meat system; water binding, gelation, solubility, swelling, , surface activity and viscosity are the most important properties in describing the usefulness of a protein and its impact on the final product quality (Table 2).

Several factors affect the functional properties of proteins (Table 3). These include the inherent properties of protein per se, whether denatured or native; the methods and conditions of isolation; the degree of purification; processing alterations, e.g. refining, drying; other components in the system and enviromental conditions (temperature, pH, salt type and concentration), all these interact to provide the final functional properties (Pour-El, 1981).

The literature is replete with papers describing the functional properties of proteins as measured by a variety of methods under differing conditions. The tendency has been for each investigator to develop methods and/or conditions to suit a particular situation with limited concern for an experimental design which would facilitate the explanation of the physical property studied. Much of the published data are of very limited value for comparative or practical purposes (Pour-El, 1981).

Ideally, equilibrium conditions should be attained when measuring the functional properties so that thermodynamic criteria are met and the specific measurement reflects the physical properties of the protein

Table 2: Functions of ingredient protein in meat-based products (Kinsella, 1982).

Improves uniform, emulsion formation and stabilization
Gelation improves firmness, pliability and texture
Facilitates cleaner, smoother slicing
Reduces cooking shrinkage and drop by entrapping/binding fats and water
Prevents fat separation
Enhances binding of meat particles without stickiness
Improves moisture-holding and mouthfeel
May impart antioxidant effects

Table 3: Some of the factors influencing the functional properties of proteins in food (Kinsella, 1982).

Intrinsic	Enviromental factors	Process treatments
Composition of protein(s)	pH	Heating
Conformation of protein(s)	Redox status	pH
Mono- or Multi-component	Salt, Ions	Ionic strength
Homogeneity-heterogeneity	Water	Reducing agents
	Carbohydrates	Storage conditions
	Lipids	Drying
	Surfactants	Physical modification
	Flavours	Chemical-enzimatic modification

under study (Shen, 1981).

1.3.2.1 Water Holding Capacity

Water holding capacity (WHC) is the ability of meat to retain water during the application of external forces such as cutting, heating, grinding, or pressing. Moreover it gives information not only on WHC of meat itself, but also on changes in the meat proteins. Changes in the charges and structure of muscle proteins markedly affect WHC. Water holding capacity is especially critical in meat ingredients that are subjected to heating, grinding, and other processes due to the weight losses that occur in the manufacturing steps (Forrest et al., 1975, pg 175).

There is no doubt that the myofibrillar proteins are primarily responsibles for water binding in muscle. The water present in the tissue is bound by different types of forces and the most accepted scheme involves 3 kinds of water, "Bound or hydratation water", "Immobilised water" and "Free or movable water"(See Figure 4). It is generally accepted that a relatively small part of the tissue water (4% to 5% of the total water) is tighly bound to the proteins as hydratation water (Hamm, 1975).

1.3.2.1.1 Influence of pH

The increase of the protein net charge by the addition of acid or base causes a loosening of the microstructure and, consequently an increase of immobilised water (Hamm, 1975). As seen in Figures 5 and 6, the pH at which the WHC is at a minimum (pH 5.0) corresponds to the isoelectric point (IEP) of the actomyosin which makes up the bulk of the

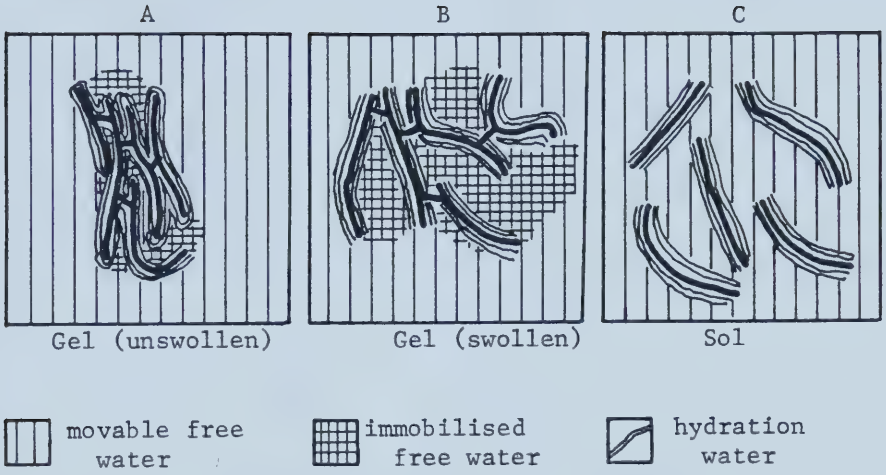


Figure 4: Influence of crosslinking of proteins or filaments on the WHC or swelling of meat.
A: Strong crosslinking causes low WHC.
B: Few crosslinks allow a high WHC.
C: No crosslinkages and, therefore, no WHC or swelling (after Hamm, 1975).

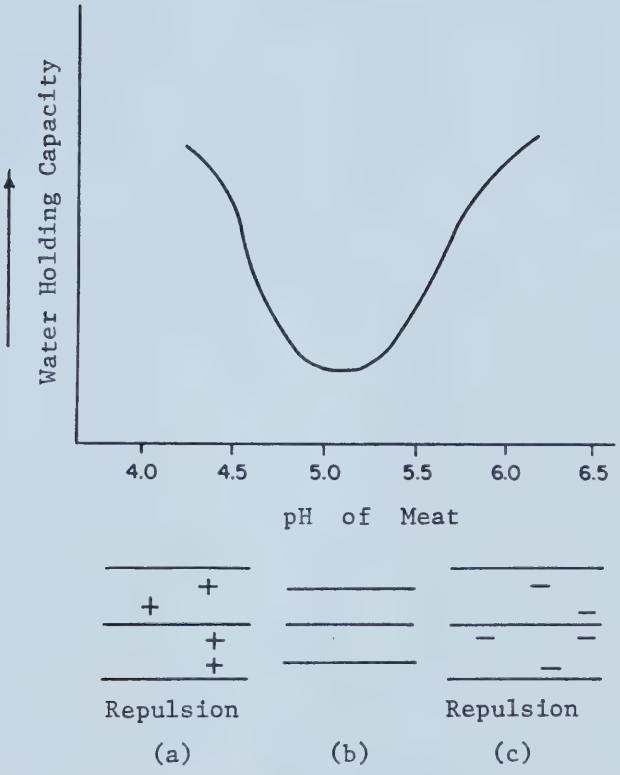


Figure 5: Effect of pH on the amount immobilized water present in meat. (a) Excess positive charges on the filaments. (b) Charges balanced. (c) Excess negative charges on the filaments (after Forrest et al., 1975).

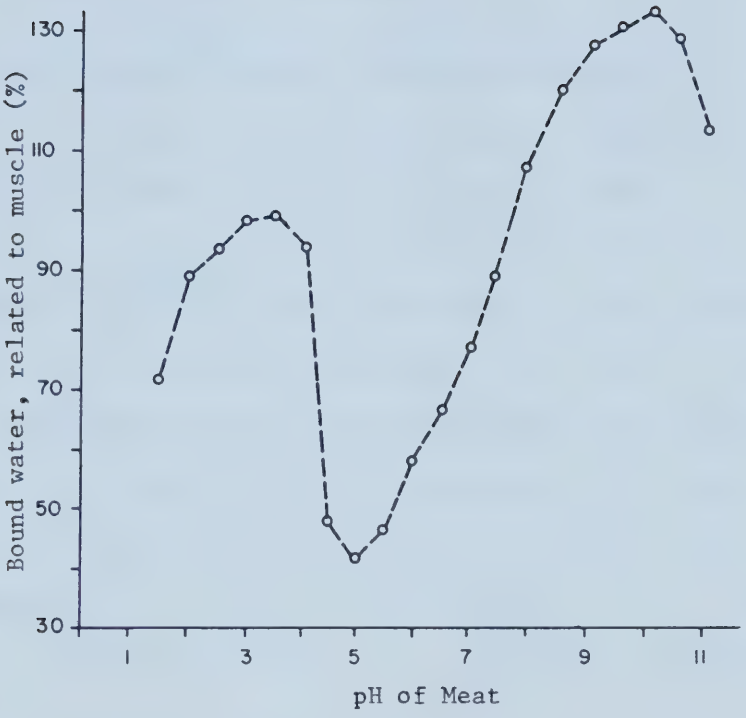


Figure 6: Influence of pH on the WHC of ground beef using filter-paper press method (after Hamm, 1975).

contractile muscle proteins. At the IEP the net charge of a protein is at a minimum; at this point we should expect a maximum of intermolecular salt linkages between positively and negatively charged groups, which explains the minimum WHC at the IEP.

1.3.2.1.2 Change of WHC during Heating

The WHC decreases considerably during the heating of meat. This results in the release of juice due to a tightening of the myofibrillar network by heat denaturation of the proteins (Fig. 4, B->A). The biggest change in total juice loss (equal to the sum of cooking loss and expressible liquid) during the cooking of beef occurs between the temperature of the raw sample and about 50 °C (Fig. 7). Karmas and Dimarco (1970) found that the heat denaturation thermoprofiles revealed two peaks at 65 and 82 °C. The protective, semi-crystalline water structure, surrounding the nonpolar amino acid radicals, may collapse as a consequence of the heat, this is followed by the formation of hydrophobic bonding which results in an aggregated, denatured state.

1.3.2.1.3 Influence of Salts on WHC

Besides the effect of pH, the presence of salts is also important to protein charges and hence to the WHC of meat. The effect of NaCl on WHC or swelling depends on the pH of the tissue (Fig. 8). Sodium chloride increases the WHC at pH values above the IEP and decreases it at pH values below the IEP (Hamm, 1975). This effect is mainly due to the Cl^- ion in NaCl, which causes a weakening of the interaction between oppositely charged groups at pH higher than IEP and a strengthening at pH less than IEP.

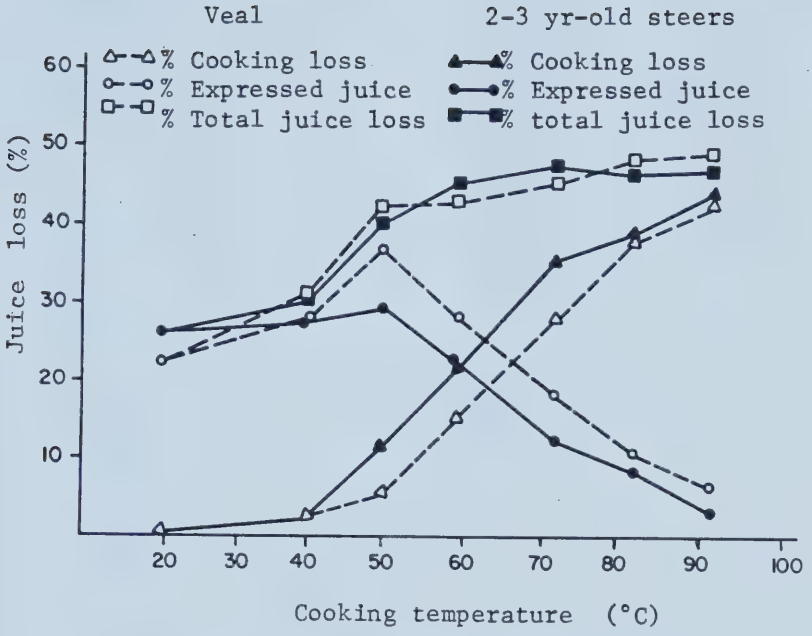


Figure 7: Changes in cooking loss, centrifugally expressible juice and total juice loss for deep pectoralis muscles from veal and 2-3 yr old steers (pH 5.4 to 5.6) cooked at different temperatures for 1 h (after Hamm, 1975).

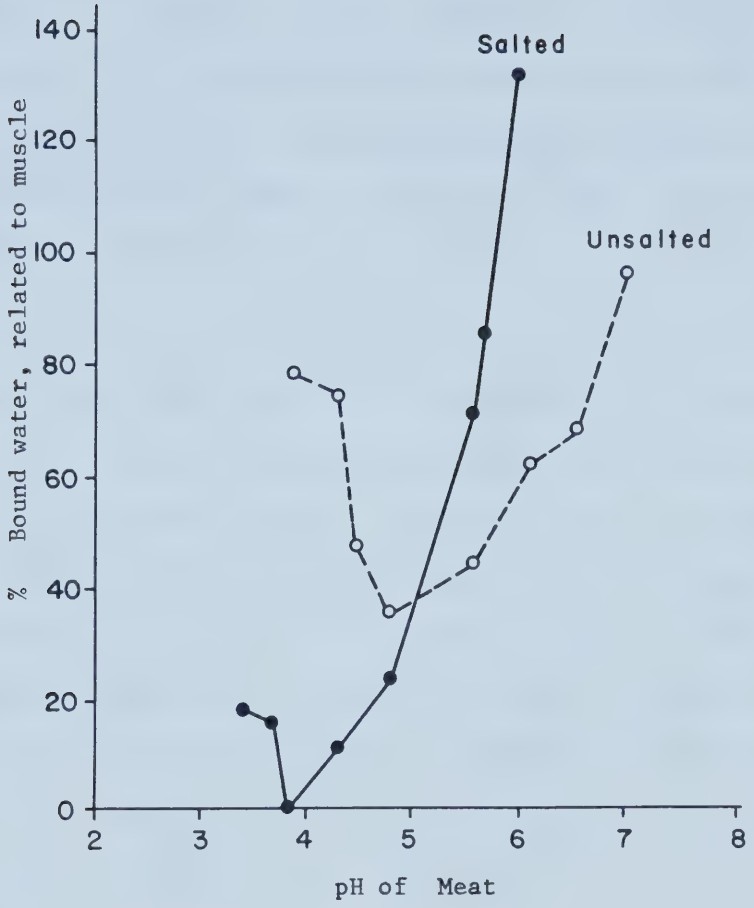


Figure 8: Influence of pH on the WHC of salted (2% NaCl) and unsalted ground beef using filter-paper press method (after Hamm, 1975).

It is in accord with this concept that, at pH values greater than the IEP, increases in ionic strength or in pH increase the electrostatic repelling forces between the muscle filaments and, consequently, the interfilamental spacing of the fibre. The work of Ohashi, Sera and Ando (1972) proposed that the addition of NaCl may lead to liberation of bivalent cations (Ca^{++}, Mg^{++}) from muscle proteins and thus a loosening of the microstructure of the tissue may take place.

When meat is minced, the sarcolemma is destroyed and the myofibrillar system is transformed from one of limited swelling to another of unlimited swelling, whereby the WHC is increased (Hamm, 1975). Most of the factors which improve the WHC of meat also increase the distribution of fat in the emulsified product. The WHC in the cooked emulsion is better when the coagulated network of proteins is larger and can fix more fat without coalesence. Prerigor meat has high WHC and it results in sausages of excellent quality because of the presence of ATP which helps to dissociate actin and myosin.

1.3.2.2 Emulsion Capacity

During manufacture of sausage and bologna, fat should be uniformly dispersed throughout the product in emulsified droplets and the emulsion should be stable to heat, and form a gel on cooking. During mixing, the water and salt soluble proteins in the meat coat the fat droplets to form a membrane. The occurrence of nonemulsified fat in emulsion type products is often a problem to the processor (Kinsella, 1982).

The extent of fat incorporation into a stable emulsion depends on

several factors including temperature, fat particle size, pH, amount and type of soluble proteins and emulsion viscosity. The amount of fat emulsified per unit of protein is referred to as Emulsion Capacity (EC) Several researchers report that myofibrillar proteins are the main emulsifying agents in meat emulsions and the optimum conditions for formation of protein films is high ionic strength at the isoelectric point (Schut, 1976).

The emulsion capacity depends partially on the shape and charge of the protein molecules and it seems that the asymmetrical shape of salt soluble proteins increases EC (Carpenter and Saffle, 1965).

A high correlation (r=0.94) was reported between the amount of soluble protein from different meat sources and the emulsifying capacity of their extracts. Freezing and cooking altered both the solubility of the proteins and their emulsifying capacity (Gillett et al., 1977). It is widely accepted that prerigor meat has a higher EC than postrigor meat, however the effect of electrical stimulation on EC has not been well elucidated (Whiting et al., 1981; Terrel et al., 1982).

The factors that may affect the EC of meat proteins in a model system are equipment design, shape of container, speed of blending, rate of oil addition, temperature, pH, protein (source, solubility and concentration), kind of oil, salt (type and concentration) and water content (Saffle, 1968). In our determinations all these variables are held constant, except that the protein functionality is expected to vary with the distinctive processes.

1.3.2.3 Binding Properties

The meat processing industry has been interested for many years in the binding abilities of meat proteins. Most research in this field has been directed towards the binding involved in meat patties and sausage products (Schmidt and Trout, 1982).

In sausage emulsions, the term bind includes water retention capability and the ability to emulsify fat. Effective binding is essential to retain the structural integrity of products during their handling and slicing.

1.3.2.3.1 Water Binding Capacity

The myofibrillar proteins have been identified as the important ones with respect to water binding capacity (WBC). However, there is some evidence that both the sarcoplasmic and the stromal proteins may also contribute to the binding characteristics of meat (Schmidt and Trout, 1982). Myosin, accounting for approximately 35% of muscle proteins, is an essential binding and gelling component and actomyosin is very important for the viscoelastic properties of the gel system. Myosin confers superior adhesive properties as compared with actomyosin at salt concentration up to 1 M (Kinsella, 1982). Because salt plays a major role in this process some workers conclude that ionic interactions are very important in the binding phenomenon. However, the principal role of salt is to deaggregate, solubilize and extract the myofibrillar proteins (Kinsella, 1982).

Collagen, the connective tissue protein, can contribute to water binding during sausage mixing or emulsification. However during heat

processing, collagen shrinks and partially gelatinizes. Shrunken and gelatinized collagen has a good water binding capacity, but is poor in fat emulsification ability (Kinsella, 1982). It has been shown that at 60 °C there is a large increase in the amount of water bound due to swelling of the collagen, but at 90 °C the amount of water bound is at the lowest level (Chien, 1975).

1.3.2.3.2 Binding Strength

The muscle proteins are required for functions besides emulsification, water binding, and emulsion stability as they should also provide the major structural properties of the cooked product. This characteristic is related to the structure-forming properties of the proteins. Once formed, the meat emulsion system must remain stable during the stresses it undergoes during subsequent operations (Randall, 1978).

Although different types of meat products including emulsions, particulate and restructured meats vary greatly in method of preparation and meat particle size, they do have one common characteristic. This is the ability to bind meat particles together to form a cohesive product. The strength of this binding is important because it determines, to a large degree, the quality of products. Binding strength is defined as the force per unit cross-sectional area required to pull apart (either directly or indirectly) the bound pieces of meat. As such, it includes a measure of both the cohesive force exerted between the binding matrix and the meat pieces and the strength of the binding matrix itself (Schmidt and Trout,1982).

The complex mechanism of binding in meat systems is not fully understood. However, the main factors that determine the efficacy of binding are protein extraction, mechanical treatment (cell disruption), presence and concentration of added salts and temperature of heating (Schnell et al., 1970; Vadehra and Baker, 1970; Schmidt et al., 1981). These may not be the only factors which determine binding ability, but they are the ones that are generally accepted as being the most important.

One way to measure binding strength (BS) is using the Kramer shear technique. This test measures the force required to pass one or more flat-edged blades through the sample (deMan et al., 1976). Voisey et al. (1975) found that of all the texture factors studied in emulsified meat products, this rupture of the sausage piece showed the best correlation (r=0.89) with the sensory attributes of chewiness and firmness.

1.4 Microbiological considerations

Emswiler and Kotula (1976) and McMillin et al.,(1981) reported little difference in the microbial quality of ground beef processed from hot and chilled beef carcasses. Studies indicate that the bacterial flora of hot processed and/or electrically stimulated meat is not different from that of conventionally processed meat (Kastner, 1982).

1.4 Statement of the Problem

Because of the economic and technical advantages of hot processing and electrical stimulation , it is necessary to know more about how these processing methods affect the properties of the meat and the protein functionality in sausage making.

The purpose of the research reported here was to measure the important functional properties in beef meat subjected to different processes, and also to measure the important functional properties of cooked emulsion products made from this meat. The results of these measurements, when correlated, should reveal the major effects of the processes, and provide some insights into the most desirable conditions for use of these new techniques.

2. MATERIALS AND METHODS

2.1 Meat Samples

Twenty culled cows, eight to twelve years old of mixed breeding, were obtained from the University of Alberta Farm. Twenty four hours after transportation the animals were stunned with a captive bolt pistol and slaughtered in the facilities of Capital Packers, Edmonton. After slaughter sides were randomly assigned to one of four carcass treatments.

2.1.1 Postmortem Treatment of Samples

Control(CB). Ten sides from different animals were chilled at approximately 4 °C until 24 h postmortem. At that time ten short plates one from each side were excised and trimmed free of visible fat. Two of them were evaluated at 24 and three at 72 h. The rest were vacuum packed in Cryovac bags and frozen at 24 h (two) and 72 h (three) in a walk in freezer (- 10 °C) for subsequent evaluation (after three weeks) as Frozen Control (CBF).

Electrical Stimulation (ES). Electrical stimulation was applied to six sides from different animals at 35-40 min postmortem. One stainless steel probe was inserted 15 cm deep at the shoulder blade of the front leg, and the rail from which the animal hangs was used as the other contact, through the shackle. Electrical stimulation consisted of a pulsed (2 sec on, 2 sec off), 475 volt, 60 Hertz, and approximately 1.5 amp alternating current for 90 sec with a Koch-Britton Stimulator 350 device. The sides were chilled at approximately 4 °C until 24 h postmortem, at which time three short plates were excised and trimmed. Each was vacuum packed in a Cryovac bag, stored at 3 °C and evaluated at

24, 48, 72 h postmortem. These will be refered to as CBES samples.

Hot Boning (HB). Hot boning was performed on the short plate muscles 1.5 h postmortem at 4 °C. Three hot boned short plates from different sides of different animals were trimmed, vacuum packed in Cryovac bags and kept at 3 °C. The tests for each of the three short plates were carried out at 24, 48,and 72 h postmortem.

Electrical Stimulation and Hot Boning (HBES). Sides were electrically stimulated as were ES sides and three short plates were hot boned, vacuum packed, stored and evaluated as previously described for HB.

2.2 Preparation of the Meat Emulsion

The first step was to develop a stable, binder free, meat emulsion. After several attempts the following formula and method of preparation were adopted.

SAUSAGE FORMULA

Lean Meat	65.70 %
Fat	18.00 %
Ice	12.00 %
Salt	3.00 %
Sugar	1.00 %
Spices	0.23 %
Erythorbate	550 ppm
Nitrite	125 ppm

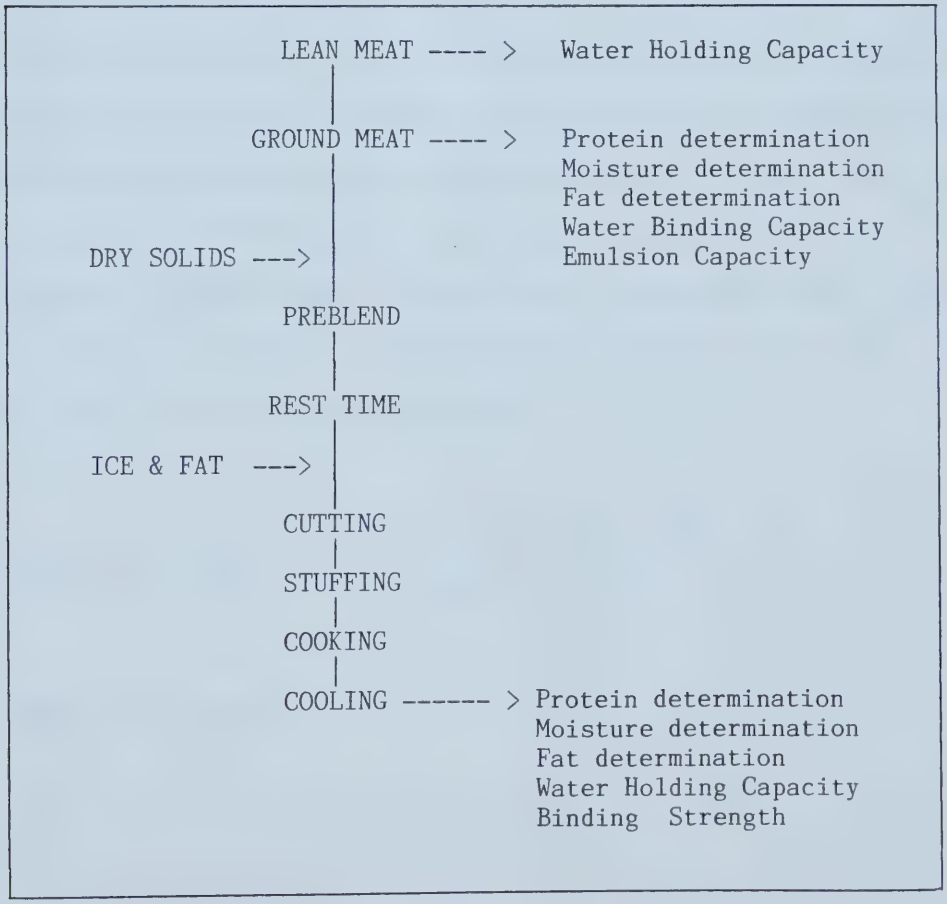
The meat was trimmed free of visible fat and ground in a 5mm plate grinder. Fat and connective tissue were also ground using the same plate. A preblend was made with the meat and the dry solids in a Omnimixer, at the lowest speed for 4 min, the mix was then left at -10 °C for 40 min. The mix was transferred to the cutter for 4.5 min and at

the second and third min the fat and ice were added, respectively. The initial and final temperatures were between 6 and 11 °C.

The uncooked mix was stuffed into glass tubes (25 mm. internal diameter and 2 mm wall thickness) fitted with rubber stoppers at the bottom. The emulsion was then heated in a water bath at 80 °C for 60 min permitting the center of the tube to reach 70 °C.

The experimental design developed to carry out the determinations is shown in Figure 9.

Figure 9: Experimental design for determinations.



The meat cut used in all cases was the short plate as it is one of the cuts commonly used in sausage making or for ground meat.

2.3 Water Holding Capacity

The method used to measure the WHC of uncooked meat and sausages was suggested by Ritchey and Hostetler (1964). Approximately 2.0-2.5 g samlple of meat or sausage was placed on a piece of aluminum foil smaller than the pressed sample would be; a second piece of foil, same size as first, was placed on top of the sample. The sample enclosed by the two pieces of foil was placed between two filter papers (Whatman #3) which were placed between plexiglass sheets (12 X 12 cm) and pressed at 1000 psi for 15 sec, with a Carver laboratory Press, allowing the expressed fluids to be absorbed by the filter paper. On removal from the press the two pieces of foil, with the thin layer of meat inside, were pulled apart, and the pressed meat was scraped into a previously dried and weighed aluminimum dish. Determinations were made for two different muscles (Serratus ventralis and Transversus abdominis) in the same area as well as for the cooked emulsion.

$$W.H.C.\% = 100 - \frac{\text{Initial (g)- Final weight (g)}}{\text{Amount of moisture (g)}} \times 100\ \%$$

2.4 Emulsion Capacity

The method used to measure emulsion capacity (EC) is actually a modification of the original system of Swift et al. (1961) involving addition of oil to dilute protein suspensions. Several variables influence the amount of oil that can be emulsified by soluble protein.

The amount of soluble protein in the original aliquot used, the speed of mixing and the final temperature of the emulsion each influence the emulsifying capacity of the soluble protein.

2.4.1 Preparation of meat slurry

A modification of the method suggested by Borton et al. (1968) was use to prepare the meat slurries. Approximately 20 g of ground meat were weighed and mixed in a Waring blendor, together with enough 1.0 M NaCl to complete 200 ml of slurry, for three bursts of 15 sec with 45 sec between each burst. The slurry was then diluted with more saline solution to a given protein concentration, approximately 3.6 mg total protein/ml slurry, and placed in a water-ice bath during the time of measurement. From the 3.6 mg total protein/ml slurry solution, solutions of approximately 1 and 2 mg/ml were prepared by dilution with 1.0 M NaCl. All the steps were performed at room temperature. The pH of the diluted slurry was measured using the Fisher Accumet pH meter equipped with a glass electrode. Unless otherwise stated, the EC of these slurries was measured 30 min after the blending was completed.

2.4.2 Measurement of Emulsion Capacity

Under standard conditions, 30 ml of diluted slurry was poured into a 400 ml beaker. A Fisher Stedi-Speed stirrer equipped with a four bladed propeller (6.5 cm diameter) was used to form the emulsion. The speed of the mixer was set at 1500 rpm using the control unit provided. Two copper electrodes with a constant distance of 2 cm between them were fitted beside the blade of the propeller. The oil was delivered by a Buchler Polystaltic pump as close to the propellers as possible via a

piece of Tygon tubing. The emulsion breakdown end-point was revealed by a sudden increase in resistance as indicated by the Avometer 8 ohmmeter. After a few seconds of mixing, the addition of rapeseed oil (Canola West) was started at a rate of 6-8 ml/min until the emulsion broke (Figure 10). Three different concentrations of slurry were tested and duplicate determinations were performed for each dilution.

2.5 Water Binding Capacity

The method of Tsai and Ockerman (1981) that measures WHC was modified to see how the heat and extraction of salt soluble proteins affects the water uptake and binding ability of the meat. For each sample of raw ground meat (six replicate determinations) 1.5 to 2.0 g were added to 10 ml of 1.0 M NaCl solution in a previously weighed 30 ml glass tube, stirred well, and heated to a temperature of 85 C for 5 min in a thermostatically controlled water bath. After heating, the samples were cooled in a water-ice bath for 5 min. The tubes were then centrifuged at 3000 rpm for 15 min. The tubes were drained and weighed again. The ratio of the final and initial weight times 100 % gave the percentage of water absorbed.

2.6 Binding Strength

To accomplish this determination the Kramer shear technique, in which one or more flat edge blades are forced through the cooked emulsion, was used. The method was suggested by deMan et al.(1976). The uncooked emulsion was stuffed into glass tubes (25 mm internal diameter and 2 mm wall thickness) and fitted with rubber stoppers in both ends. The emulsion was then heated in a water bath at 80 C for 60 min.

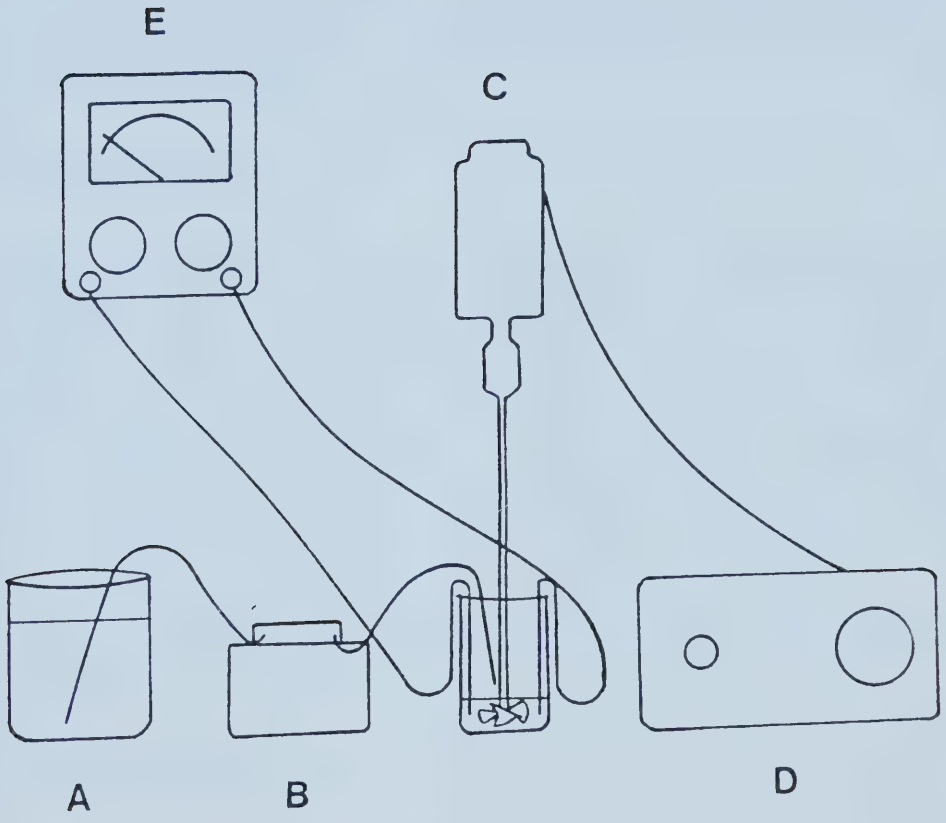


Figure 10: Experimental set up for EC measurement
A: oil supply
B: pump
C: Stedi Speed stirrer (motor)
D: Stedi Speed stirrer (control unit)
E: ohmmeter
(after Ouellet, 1979)

The samples were held in a cool room (at 4 °C) for 24 h. The samples were then allowed to reach room temperature before shear determination. A cylindrical piece from the middle of the glass tube with the same length as the Kramer cell was taken, and placed in the cell. The cross head speed was set at 5 cm/min, the speed recommended by Huang and Robertson (1977). The maximum force require to pass the blades through the sample was recorded.

2.7 Proximal Analysis

Determinations of moisture, fat and protein were performed on all the samples. Moisture was determined using the air oven method as suggested by the American Meat Institute and fat was measured using the Soxhlet extraction as proposed by United States Department of Agriculture. When the protein content of ground meat and cooked emulsion was determined, the procedure of Torten and Whitaker (1964) was used. This method included alkaline digestion of the sample and the use of the Biuret method.

2.8 Statistical Analysis

The statistical analysis was done using the SSPSSx statistical package from SPSS Inc. One way analysis of variance was performed on the experimental data and Tukey's test (Honestly Significant Difference) was utilized for multiple comparisons between means at the $P<0.05$ level.

3. RESULTS AND DISCUSSION

The presentation and discussion of results have been divided into two main parts. The first part deals with the different functional property determinations conducted on the samples and the second part is dedicated to analyze how these functional properties are affected by the different processes studied.

3.1 Proximal analysis

Determinations of protein, fat and moisture were performed in raw and cooked samples (Tables 4 and 5). Statistical analysis of the results for both raw and cooked samples revealed that all samples were homogeneous ($P<0.05$) with respect to protein, fat and moisture content. However, the fat and moisture content within the process categories showed moderate but nonsignificant variations. The variations in the batches for the same processes could be attributed to such factors as biological and environmental differences, the amount of untrimmed fat in the muscle, the amount of the water evaporated postmortem, or some combination of these.

3.2 Determinations in Raw Meat

3.2.1 Water Holding Capacity

As can be seen in Table 6 and Figure 11, as postmortem time increased to 72 h the values for WHC decreased. This decreasing trend in the muscle WHC may be due to degradative processes that are taking place during the early stages of aging of the meat. The degradative processes are thought to be enzymatic in nature, with the most

Table 4: Proximal analysis of raw meat.

PROCESS	PROTEIN* %	MOISTURE* %	FAT* %	TOTAL
CB	18.5 (0.4)	75.2 (0.6)	4.8 (1.1)	98.5
CBF	18.2 (0.3)	76.0 (1.0)	2.8 (1.7)	97.0
CBES	18.7 (0.4)	75.9 (1.5)	2.9 (1.4)	97.5
HB	18.2 (0.4)	75.5 (1.5)	3.1 (1.3)	96.8
HBES	18.5 (0.5)	75.4 (1.8)	3.2 (1.2)	97.1

*Arithmetic means for 9 replicates (Standard deviation)

Table 5: Proximal analysis of cooked emulsions.

PROCESS	PROTEIN* %	MOISTURE* %	FAT* %	TOTAL
CB	14.3 (0.6)	62.1 (0.6)	14.6 (0.7)	91.0
CBF	14.2 (0.3)	62.6 (1.5)	14.7 (1.1)	91.5
CBES	14.8 (0.6)	63.8 (1.8)	14.2 (1.3)	92.8
HB	15.0 (0.6)	63.9 (1.0)	15.0 (0.9)	93.9
HBES	14.2 (0.6)	64.3 (0.8)	13.9 (0.8)	92.4

* Arithmetic means for 9 replicates (Standard deviation)

Table 6: WHC* of raw meat (%) at three postmortem times.

PROCESS	POSTMORTEM TIME (h) 24	48	72
CB	78.6 [5.8](2.4)	-	78.2 [5.9](2.2)
CBF	60.9 [5.8](3.0)	-	57.2 [5.9](0.8)
CBES	77.1 [5.7](1.0)	74.2 [5.7](2.2)	73.4 [6.0](0.4)
HB	74.2 [6.0](1.0)	71.5 [5.8](0.9)	70.9 [5.9](0.7)
HBES	80.2 [6.0](0.7)	76.8 [5.8](1.5)	75.4 [6.0](0.9)

* Arithmetic means of 8 replicates [pH of meat] (Standar deviation)

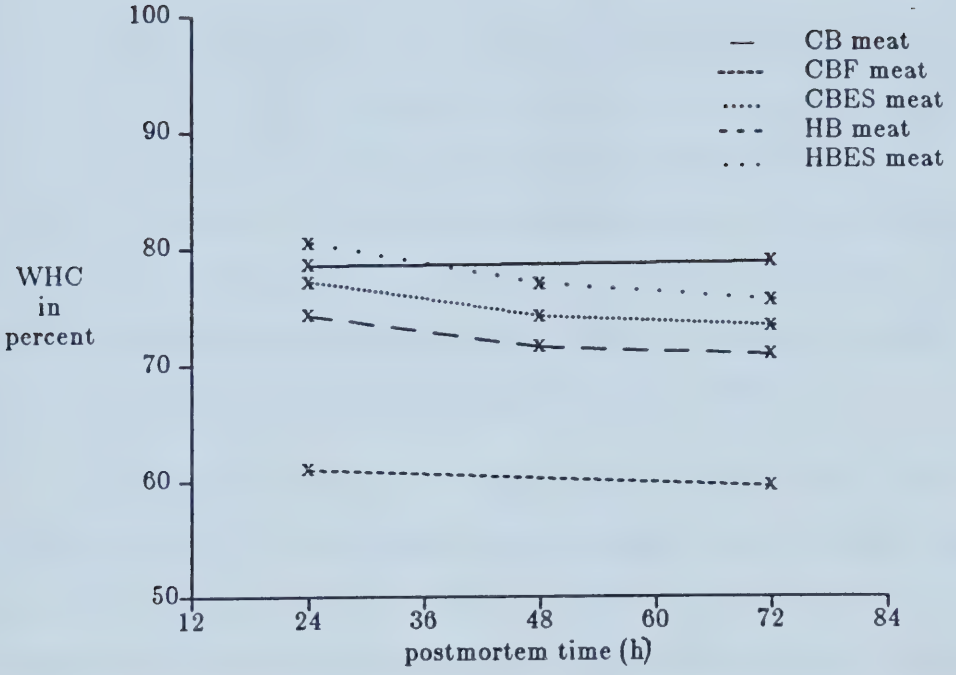


Figure 11: Water Holding Capacity of Raw Meat at three Postmortem Times.

significant being degradation of protein and a consequent alteration of membrane permeability (Forrest et al., 1975, pg 178), however protein denaturation and cell integrity may play an important role (Tarrant, 1982). The pH at the time of WHC determination was near to the ultimate pH. As can be seen in Table 6 pH differences between samples were small, and did not have a marked effect on WHC.

At any of the postmortem times studied CBF muscle had the lowest WHC ($P<0.05$). This indicates that freezing causes more damage to the muscle structure and affects the associated WHC more than the other processes examined. The main contributing factors to this situation are probably the damage to the sarcolemma caused by ice crystals and the protein denaturation and aggregation that occurs during freezing (Awad et al.. 1968; Jimenez Colmeneros and Borderias, 1983).

Cold boned meat has a higher WHC ($P<0.05$) than CBES meat, indicating that ES effectively decreases WHC. Savell (1982) suggested that the rapid decline of pH in CBES muscle, while the muscle temperature is still high, is similar to the PSE condition in pork which also results in reduced WHC. In the case of HB meat our results show that ES increases WHC ($P<0.05$) at all postmortem times. This opposite situation as compared to CB may be due to less severe shortening of muscle fibers during postmortem conditioning and this observation is in accord with the studies of Taylor et al. (1980).

Cold shortening can occur in the carcass; however, hot processed prerigor muscle may be more susceptible to that effect than cold boned meat. Excised prerigor muscle can freely contract in the absence of skeletal restraints (Kastner, 1982), compressing the sarcomere structure

(Schmidt and Keman, 1974) and irreversibly fixing the myofilaments in the high overlap (contracted) position (Tarrant, 1982). It seems contradictory that the structural damage and related protein degradation (caused by electrically induced contractions) and faster pH fall can decrease the WHC in the carcass attached muscle (CB) and increase the WHC in the unattached muscle (HB). This suggests that the control over muscle fiber shortening produced by ES in the HB meat has a greater effect on WHC than protein and structural degradative effects (Rashid et al., 1983). This is corroborated by the fact that CBES and HBES meats are not significantly different ($P<0.05$) at the three postmortem times analysed.

3.2.2 Water Binding Capacity

Water binding capacity (WBC) is a general term used to describe the extent to which water is held by meat or meat products once cooked. At the molecular level, WBC seems to be determined by the formation of a characteristic three-dimensional lattice stucture. This is the result of the unfolding of the alpha-helical rod portion of myosin followed by intermolecular crosslinking (Trout and Schmidt, 1983). However myosin is not the only protein involved in water binding , stromal and sarcoplasmic proteins also contribute to WBC (Schimdt and Trout, 1982). As well, Chien (1975) has shown that at between 60 and 90 ^{o}C the swelling of collagen can be responsible for an uptake of 33% of the bound water. Table 7 and Figure 12 present the data obtained on WBC for the various treatments.

Table 7: WBC* of raw meat (%) at three postmorterm times.

PROCESS	POSTMORTEM TIME (h) 24	48	72
CB	69.7 (3.7)	-	68.1 (2.2)
CBF	59.0 (3.4)	-	56.3 (1.9)
CBES	49.9 (2.3)	48.2 (1.2)	52.1 (0.5)
HB	71.2 (1.5)	57.4 (2.1)	58.6 (1.6)
HBES	71.3 (1.1)	65.0 (1.2)	52.8 (1.0)

* Arithmetic means of 6 replicates (Standard deviation)

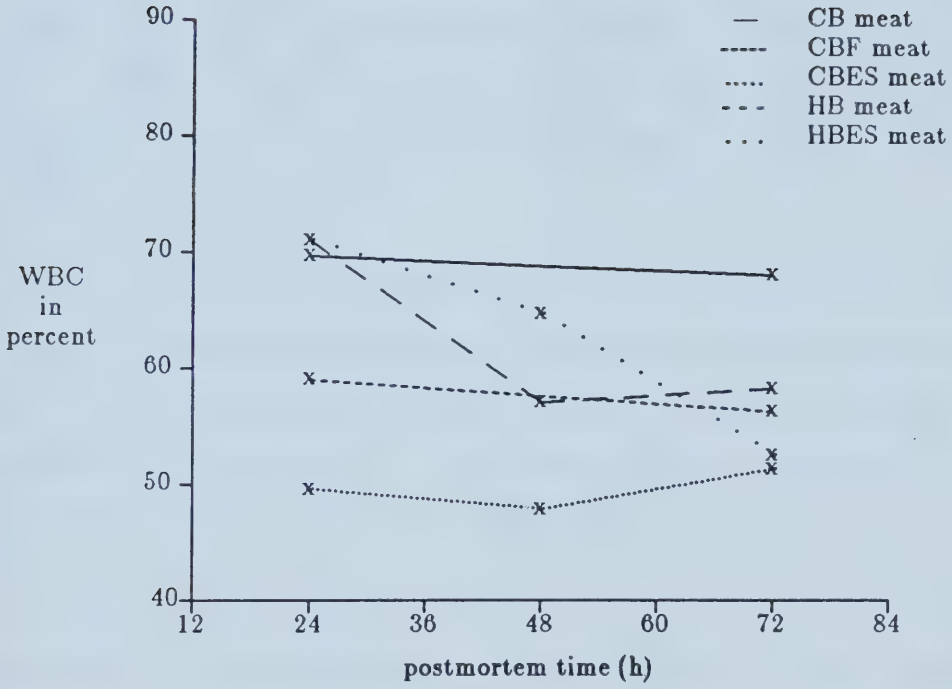


Figure 12: Water Binding Capacity of Raw Meat at three Postmortem Times.

A significant ($P<0.05$) difference in WBC values was determined when comparing CB, CBF and CBES meat. However postmortem times did not affect the WBC of any of the cold processed meats. The small variations observed with respect to time were not significant at the $P<0.05$ level. It appears that ES has a larger negative effect on WBC than freezing. The decline in WBC for CBF meat may be primarily caused by the denaturation of both sarcoplasmic and actomyosin complex proteins (Awad et al., 1968). Electrical stimulation may accelerate the denaturation by increasing the rate of postmortem pH decline. This effect is even more pronounced than freezing.

Hot boned meat (both stimulated and unstimulated) showed a decline ($P<0.05$) in WBC between 24 and 48 h. After 48 h WBC had stabilized in HB meat, but it continued to decrease in HBES, and both observations are statistically significant. This indicates that the influence of aging over WBC is greater in HB than in CB meats.

Water binding capacity depends on time of excision. However ES produced a significant ($P<0.05$) change in HB and CB meat, indicating that WBC is adversely affected by ES, freezing and time of muscle excision after 48 h. That may be due to a limited protein unfolding and probably to physical disruption of molecules.

3.2.3 Emulsion Capacity

Emulsion capacity (EC) refers to the ability of the protein to emulsify fat. Emulsion capacity was determined using meat homogenates prepared from the differently processed meats. The pH values, which ranged from 5.50 to 5.90 in the various homogenates, did not show any

significant differences ($P<0.05$), at the time of EC determination .

Different dilutions of the same homogenate were tested as can be seen in Tables 8 and 9. These results are graphically represented in Figures 13, 14, 15, 16 and 17. In all the graphs there is a clear trend showing that as postmortem time increased the EC also increased. This could be attributed to limited proteolytic effects of the naturally occurring enzymes in meat (Hay et al., 1973). As stated by Forrest et al., (1975, pg 186) limited protein degradation and dissociation of the actomyosin complex occurs in muscle with increasing postmortem time and these changes are accompanied by a fragmentation of the filamentous structure. These changes probably increased EC to a certain extent. In support of this argument, Du Bois et al.(1972) found that the treatment of muscle homogenates with proteases resulted in an increase in the extractability of salt soluble proteins and consequently higher values for EC. This indicates that aging may possibly (to a small extent) increase protein extractability, hence resulting in higher EC.

In the different processes, average values for protein concentration and emulsion capacity for each dilution were tabulated (Table 10) and are graphically represented in Figure 18. As seen in Figure 18, as the protein concentration increased the amount of oil necessary to break the emulsion increased. Between 1.0 and 2.0 mg of protein per ml of meat homogenate there was an increasing trend, but no significant differences were found among the processes. However at the highest concentration tested the differences were statistically important ($P<0.05$). This indicates that as the protein concentration increased the differences between the processes are more profound.

Table No. 8 : Emulsion capacity@ of CB and CBF at three protein concentrations.

PROCESS	POSTMORTEM TIME (h)	pH	PROTEIN CONC. *	EMULSION CAPACITY (ml of oil) 1 : 2 #	1 : 1	1 : 0
CB	24	5.70	3.5	49.5	58.5	89.5
	72	5.85	3.7	50.0	60.5	103.0
CBF	24	5.80	3.6	45.5	55.5	77.0
	72	5.75	3.6	42.5	57.5	92.0

@ Arithmetic means of 3 replicates.
* (mg of total protein/ ml of slurry)
# Ratio of slurry and 1 M NaCl in total volume of 30 ml.

Table No. 9 : Emulsion capacity@ of CBES, HB and HBES at three protein concentration.

PROCESS	POSTMORTEM TIME (h)	pH	PROTEIN CONC. *	EMULSION CAPACITY (ml of oil) 1 : 2 #	1 : 1	1 : 0
CBES	24	5.50	3.7	44.0	49.5	67.5
	48	5.60	3.8	50.0	54.0	81.0
	72	5.85	3.9	49.0	66.5	113.5
HB	24	5.60	3.9	46.0	55.0	101.0
	48	5.65	3.5	43.5	55.5	109.0
	72	5.70	3.5	38.0	66.5	117.0
HBES	24	5.80	3.7	41.0	56.0	119.0
	48	5.60	3.6	46.0	69.5	131.0
	72	5.90	4.0	47.0	65.0	150.0

@ Arithmetic means of 3 replicates.
* (mg of total protein/ ml of slurry)
# Ratio of slurry and 1 M NaCl in a total volume of 30 ml.

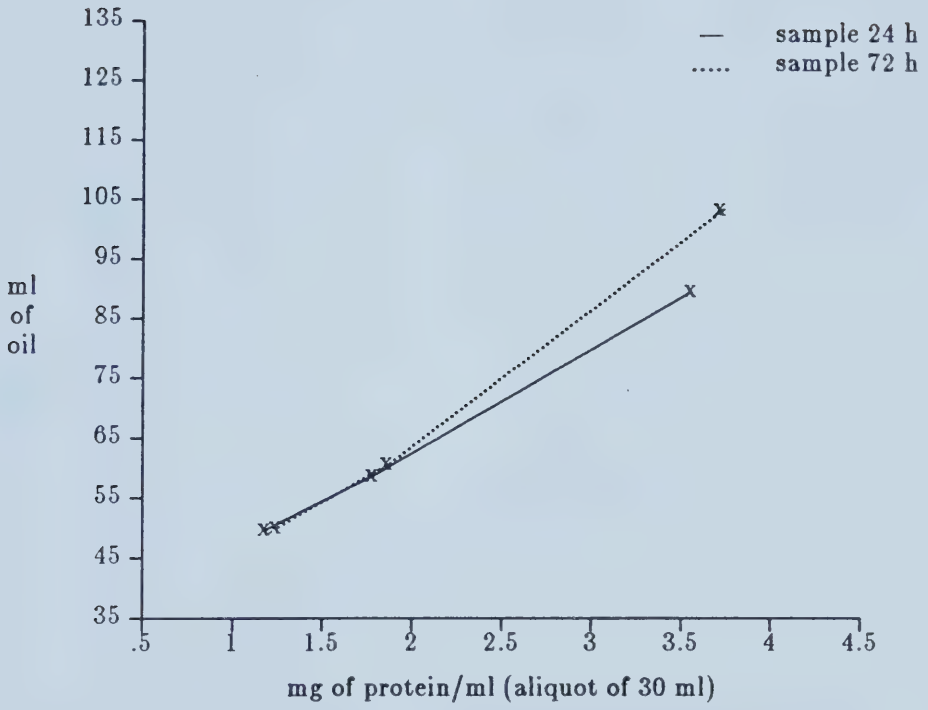


Figure 13: Emulsion Capacity of Cold Boned Meat

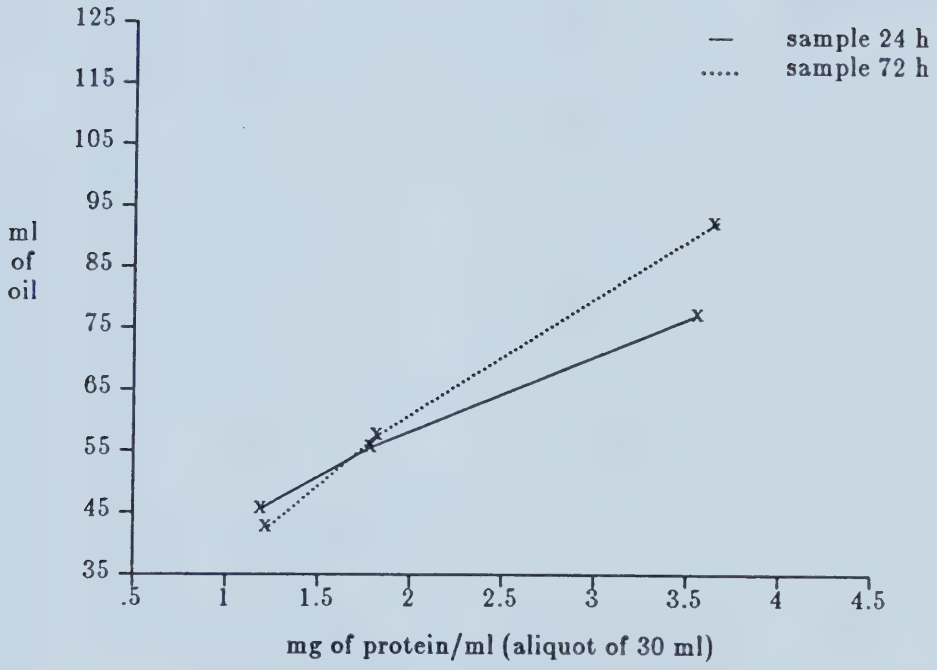


Figure 14: Emulsion Capacity of Cold Boned Frozen Meat

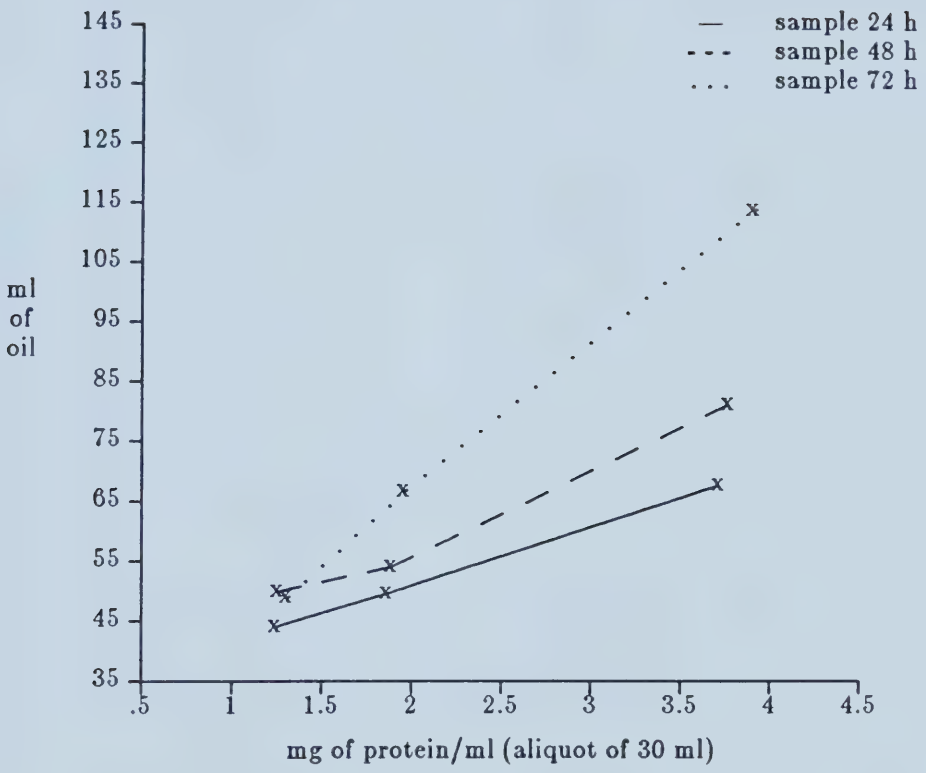


Figure 15: Emulsion Capacity of Cold Boned Electrically Stimulated Meat

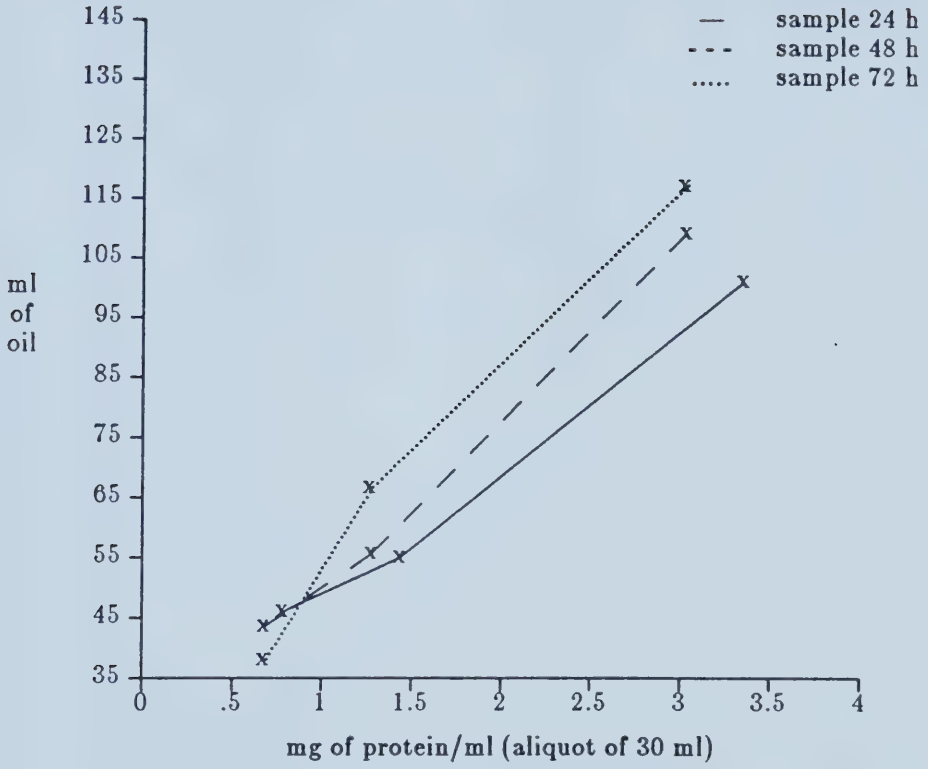


Figure 16: Emulsion Capacity of Hot Boned Meat

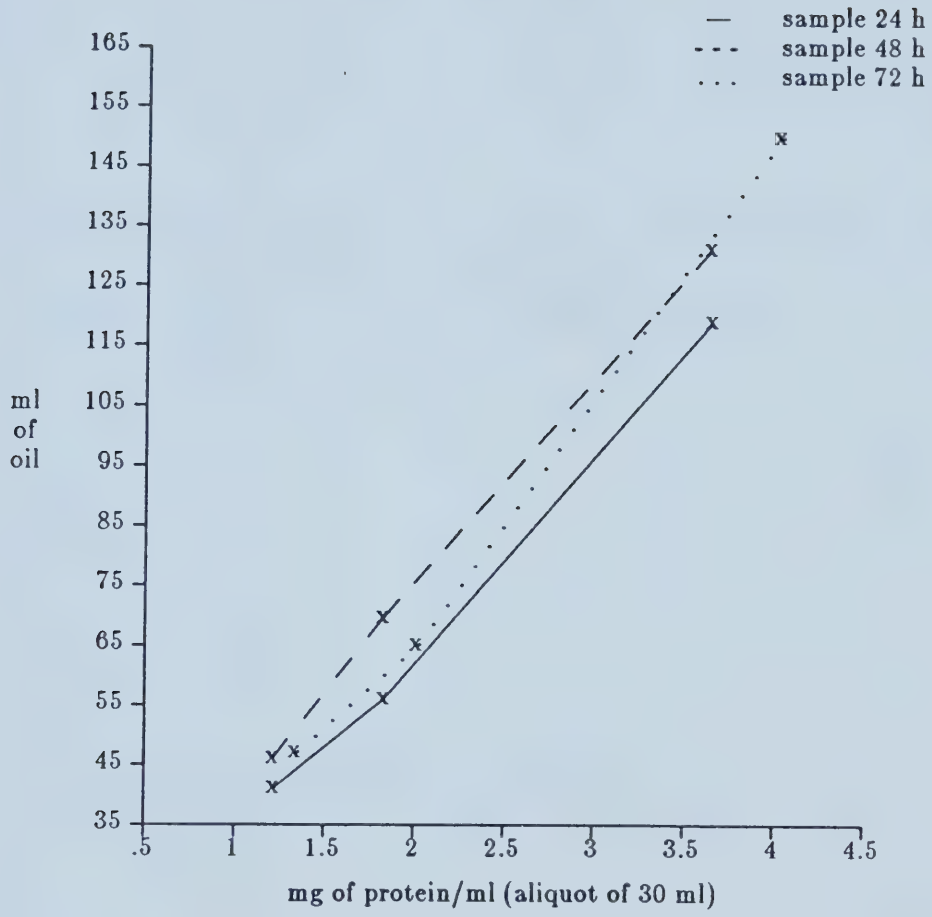


Figure 17: Emulsion Capacity of Hot Boned Electrically Stimulated Meat

Table 10: Emusion Capacity for CB, CBF, CBES, HB and HBES meats at three protein concentrations.

PROCESS	PROTEIN CONC. @	EMULSION CAPACITY 1 : 2 #	1 : 1	1 : 0
CB	3.6	50.0	59.5	96.0
CBF	3.6	44.0	58.0	84.5
CBES	3.8	48.5	57.0	87.0
HB	3.6	43.5	59.0	110.0
HBES	3.8	45.0	63.5	133.0

@ (mg of total protein/ml of slurry).
* Average values for the different postmortem times.
# Ratio of slurry and 1 M NaCl in total volume of 30 ml.

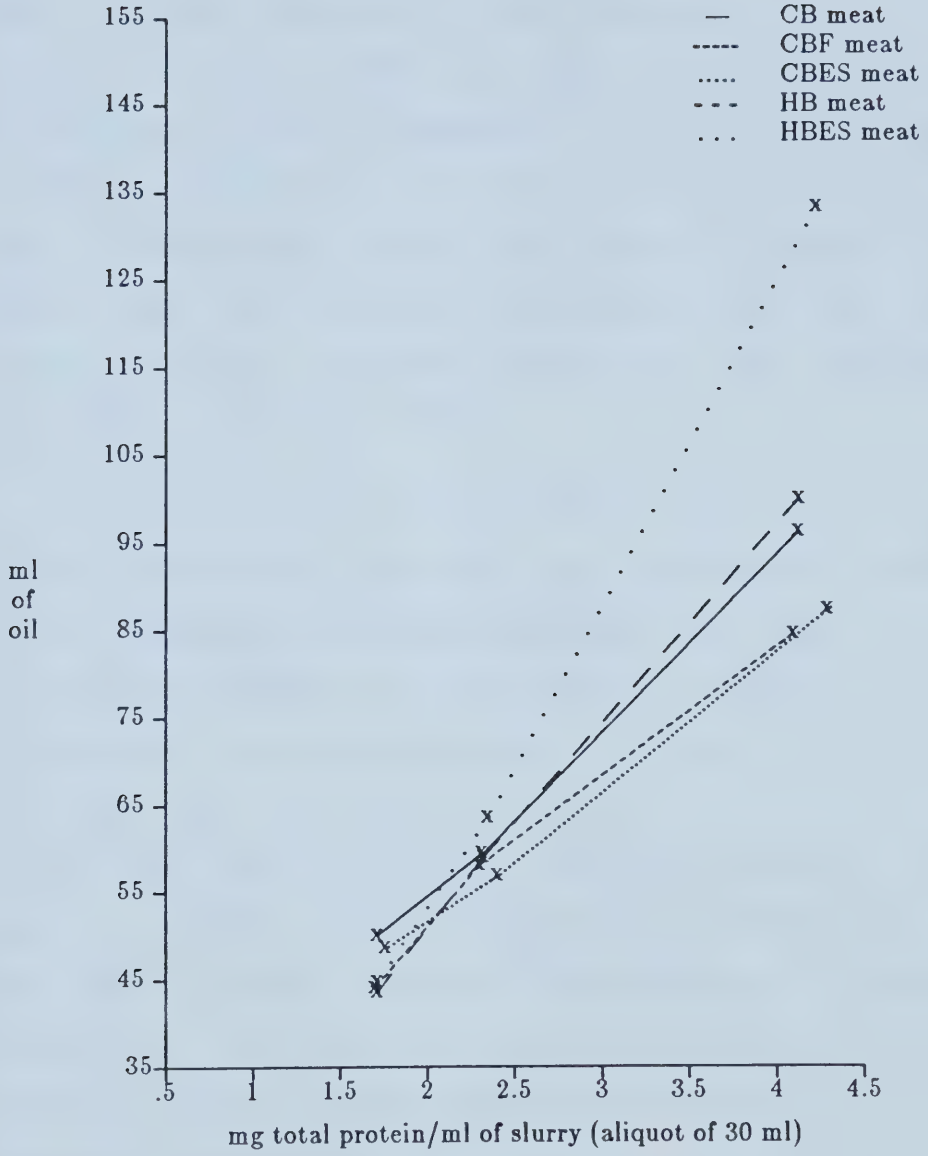


Figure 18: Emulsion Capacity of Differently Processed Meats in ml of Oil

When EC was expressed as volume of oil per unit of protein the emulsifying ability of the protein showed a decrease as the protein concentration was increased (as seen these results in Table 11 and Figure 19). Saffle (1968) attributed these decreases in EC to overloading of the system. Subsequently, Ivey et al., (1970) suggested that thicker protein membranes were found on the fat globules at higher protein concentrations, utilizing more protein per particle of fat. However, HBES seems to change the decreasing trend which has been accepted by various researchers (Swift et al., 1961; Carpenter and Saffle, 1964; Gillet et al., 1977).

The data presented in Table 11 and 12 were produced by the following calculations; the amount of oil emulsified was divided by protein concentration (mg/ml) and the volume of slurry used to perform the determination (30 ml). As seen in Table 12 and Figure 20, at any of the postmortem times studied HBES meat has a higher EC ($P<0.05$) than CBES, indicating that the excision time and the associated faster chilling rates are more important than ES in postrigor preblended samples. Furthermore, the EC arithmetic means for HB meats are always higher than for CB meats. This behavior may be attributed to the greater extractability and to a more extended physical disruption of myofibrillar proteins during conditioning of HB meat (Kastner et al., 1976; Bowles et al., 1983).

Electrical stimulation has a different effect depending on whether the samples were HB or CB. Hot boned postrigor preblends had higher emulsion capacity after ES. However, this effect was reversed when the meat used was CB. It has been suggested by Choi et al., (1984) that ES

Table 11: Emulsion capacity* for CB, CBF, CBES, HB and HBES meats at three protein concentrations.

PROCESS	PROTEIN CONC. @	ml Oil/mg Protein 1 : 2 #	1 : 1	1 : 0
CB	3.6	1.4	1.1	0.9
CBF	3.6	1.2	1.1	0.8
CBES	3.8	1.3	1.0	0.8
HB	3.6	1.2	1.1	1.0
HBES	3.8	1.2	1.1	1.2

@ (mg of total protein/ml of slurry).
* Average values for the different postmortem times.
# Ratio of slurry and 1 M NaCl in total volume of 30 ml.

Table 12: Emulsion capacity* of meat homogenates in ml oil/mg total protein at the highest protein concentration tested.

PROCESS	POSTMORTEM TIME (h) 24	48	72
CB	0.84 (0.02)	-	0.92 (0.05)
CBF	0.72 (0.03)	-	0.84 (0.01)
CBES	0.61 (0.05)	0.72 (0.13)	0.97 (0.09)
HB	0.87 (0.09)	1.03 (0.08)	1.11 (0.05)
HBES	1.09 (0.02)	1.20 (0.08)	1.25 (0.05)

* Arithmetic means of 3 replicates (Standard deviation)

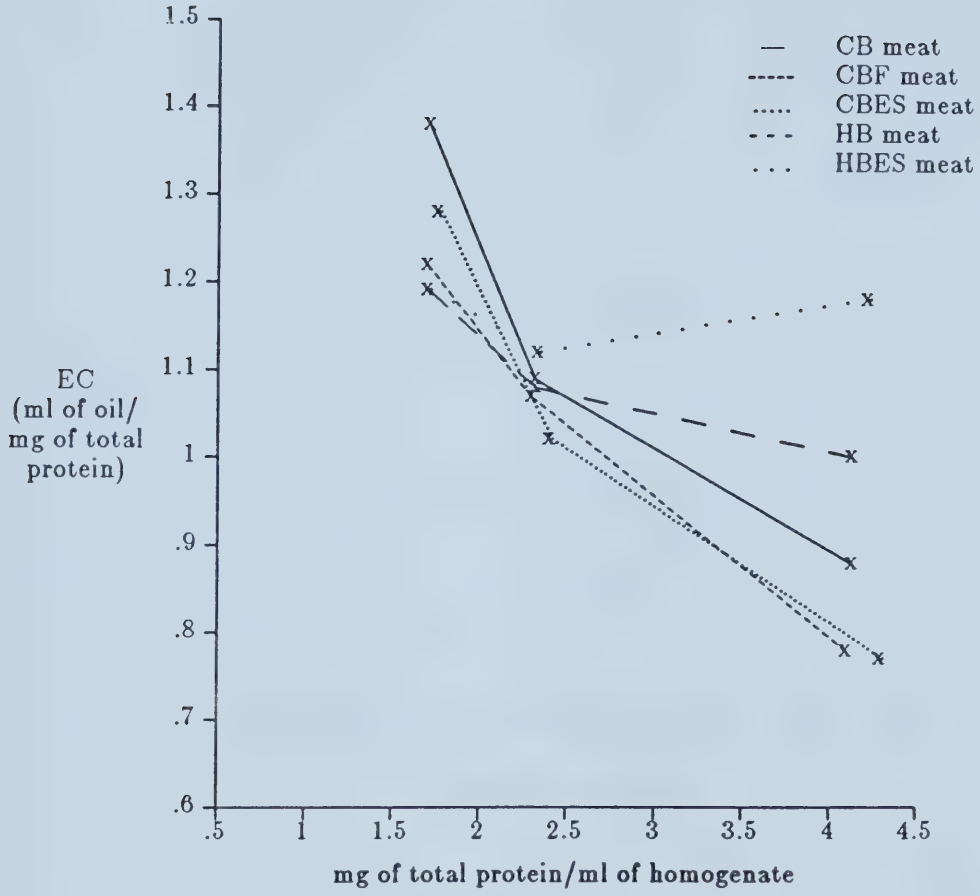


Figure 19: Emulsion Capacity of Differently Processed Meats in ml oil/mg total protein

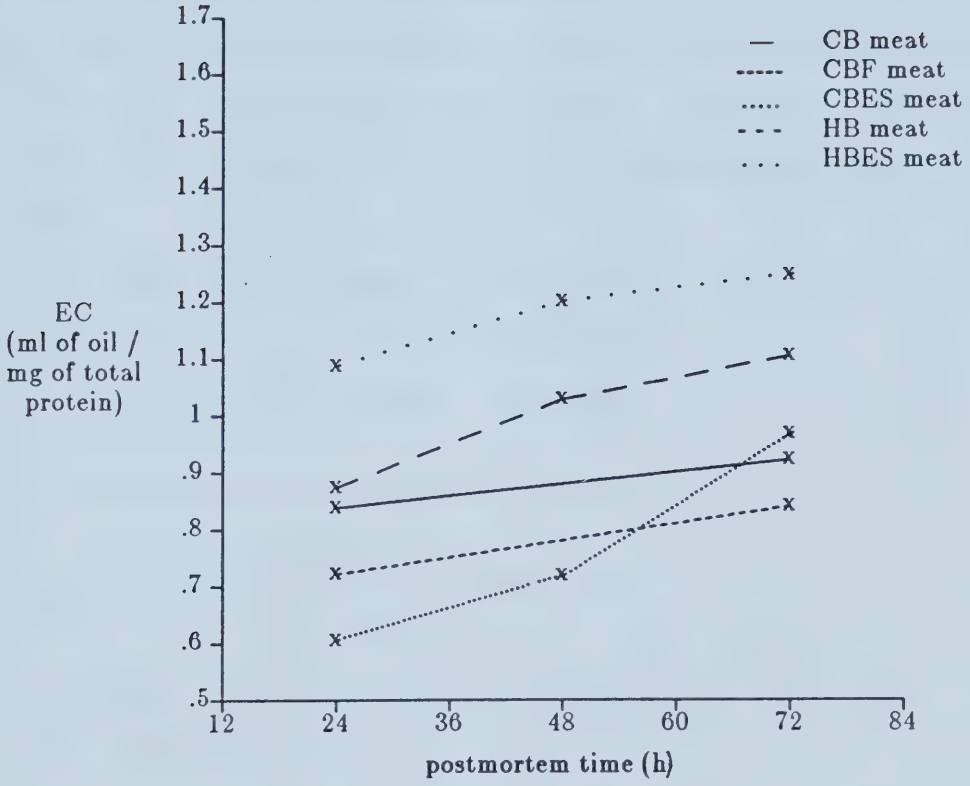


Figure 20: Emulsion Capacity of Differently Processed Meats at three Postmortem Times

may change protein solubility and decrease the EC due to the rapid pH decline after stimulation.

Cold boned frozen preblends show a lower EC than CB preblends. This change in EC could be due to the loss of protein solubility. Loss of solubility may be attributed to denaturation and protein aggregation induced by the freezing process (Jimenez Colmeneros and Borderias, 1983). In addition, according to the mechanism postulated by King (1966), some actomyosin aggregates form by reaction with fatty acids in frozen muscle. Finally, more actomyosin depolymerizes and separates into G-actin and myosin in the high ionic strength liquid phase. Subsequently both proteins may form insoluble aggregates during frozen storage.

3.3 Determinations in the Cooked Emulsion

To obtain the desirable textural characteristics in emulsified meat products, the raw material should have a good binding potential. The measurement of WHC and binding strength (BS) in the cooked product should reveal this potential.

In sausage emulsions, soluble proteins dissolve and unfold in the aqueous phase, acting as an emulsifying agent by coating all surfaces of the dispersed fat particles. Permanent stability is achieved by heat coagulation of the proteins in the aqueous phase. A three dimensional protein network holds the fat in suspension for an unlimited period of time and also holds water by capillarity (Tarrant, 1982).

3.3.1 Water Holding Capacity

Heat-coagulation causes a decrease in meat protein WHC compared to raw unground meat. Protein denaturation, and loss of the structural integrity of the meat probably operate synergistically altering the structure in which water is held.

In Table 13 and Figure 21, CB meat shows the highest WHC in the cooked state. The only significant difference ($P<0.05$) in WHC at 24 h is observed between CB and HBES meat. In emulsions prepared from 72 h. postmortem meat the differences are greater and CB meat has a significantly higher WHC ($P<0.05$) than HB, HBES and CBF. Electrical stimulation does not have any special effect over the samples. Hot boned and freezing processes seem to be responsible for the loss of WHC. Water is mainly held by capillary forces and the three dimensional protein network. This network is developed after thermal setting. It may be assumed that WHC is dependant on the strength and degree of intermolecular crosslinking of the emulsified protein network. It has been shown by Samejima et al. (1981) that intact myosin molecules are required to develop desirable gel strengths. Hot boning and freezing may affect the integrity of the myosin molecule, causing a decrease in intermolecular bonding strengths and in the associated WHC.

3.3.2 Binding Strength

The binding strength (BS) of the cooked emulsion was measured in sausages prepared at three postmortem times using the Kramer cell. The binding strength data presented in Table 14 for the three postmortem times are quite consistent and the slight deviations within the groups

Table 13: Water holding capacity* (%) for cooked emulsions.

PROCESS	POSTMORTEM TIME (h) 24	48	72
CB	61.0 (3.6)	-	62.8 (5.0)
CBF	57.2 (3.0)	-	56.1 (1.1)
CBES	56.1 (1.4)	59.3 (1.6)	59.0 (1.0)
HB	56.7 (1.2)	57.9 (1.2)	52.0 (1.0)
HBES	53.9 (0.6)	57.4 (1.0)	55.0 (1.2)

* Arithmetic means of 6 measurements (Standard deviation)

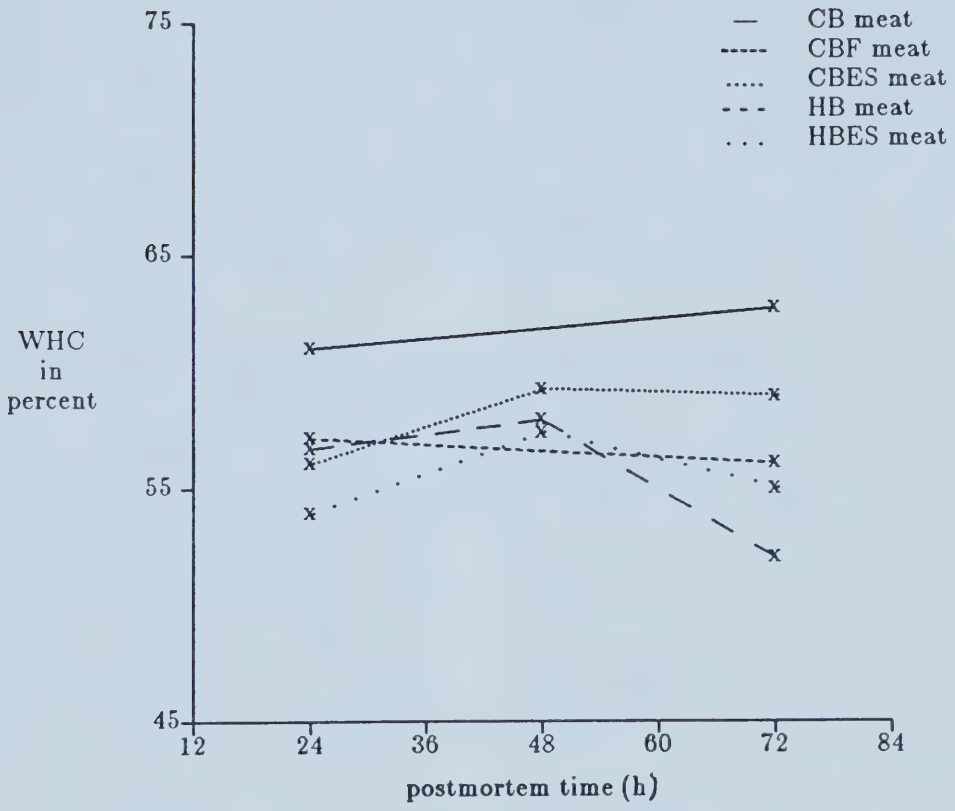


Figure 21: Water Holding Capacity of Cooked Emulsions at three Postmortem Times

Table 14: Binding strength* of cooked emulsion in arbitrary units.

PROCESS	POSTMORTEM TIME (h) 24	48	72
CB	35.0 (1.2)	-	35.3 (1.4)
CBF	28.6 (1.8)	-	29.9 (0.7)
CBES	35.8 (0.9)	35.4 (0.8)	36.6 (0.9)
HB	30.4 (1.0)	30.4 (1.3)	30.1 (1.5)
HBES	30.1 (0.7)	30.1 (0.9)	31.8 (1.0)

* Arithmetic means of 6 measurements (Standard deviation)

are not statistically significant ($P<0.05$).

This suggests that the BS of postrigor meat sausages was not influenced by the postmortem changes in the meat within the period of time studied. As reported by Lawrie (1975, pg 84), the ultimate pH in the meat is reached before 24 h. Also Valin (1982) and Bowles et al., (1983) showed that between day 1 and 4 postmortem, biochemical and physical measurements indicate that ES has no significant effect on the aging changes.

As can be seen in Figure 22 there are two ranges of values for BS. At any postmortem time the CB samples (either stimulated or unstimulated) showed a higher BS ($P<0.05$) than HB, HBES and CBF samples. This indicates that the time of muscle excision is more important than ES with respect to binding strength. Pepper and Schmidt (1975) compared beef rolls manufactured with either HB or CB beef rounds they found that HB rolls had a lower binding strength than CB rolls.

The results of this experiment suggest that the effect of freezing on BS of the cooked emulsion is comparable to HB. However, the changes induced by HB or freezing at the salt-soluble protein and meat structure levels may be different.

Myofibrillar proteins have been implicated as being the most important proteins involved in binding. Samejima et al., (1981) showed that the integrity of the myosin molecule is fundamental in the ultrastructure of the gel and in development of the binding strength. In fact molecules of myosin alone produced a three dimensional network system and gels of adequate firmness. Fragmented molecules of myosin

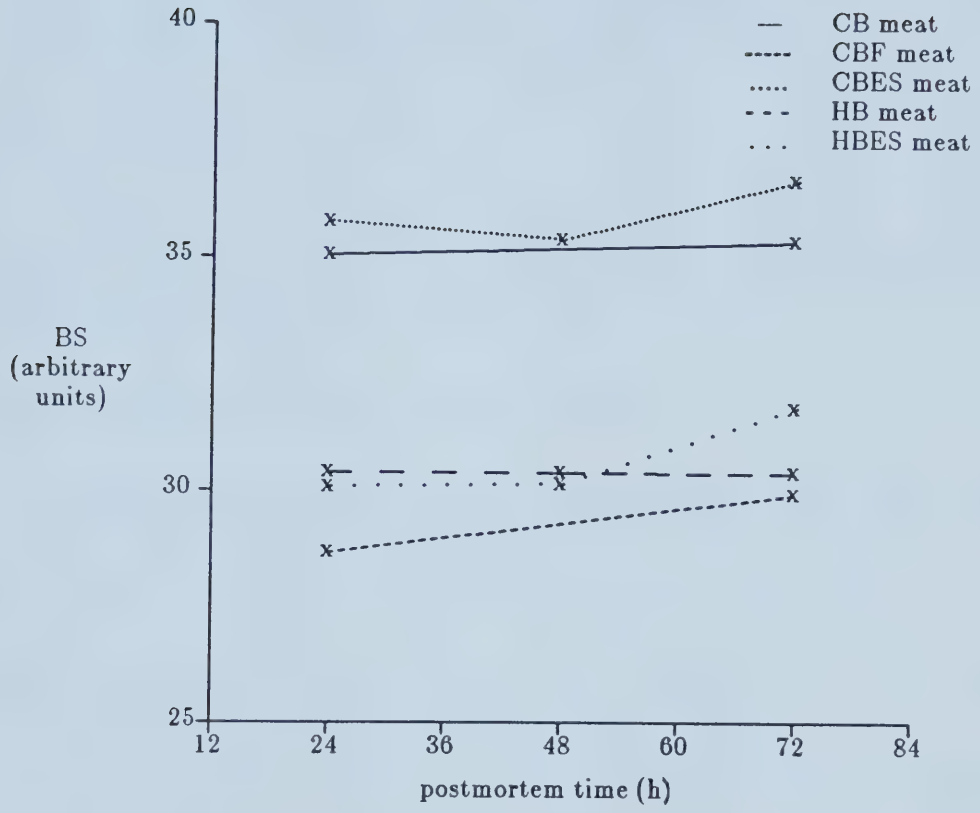


Figure 22: Binding Strength of Cooked Emulsions at three Postmortem Times

produced weak gels and particulate aggregation upon heating. However, not only myosin is involved in intermolecular binding, sarcoplasmic and stromal proteins may contribute significantly to the binding characteristics of the meat (Schmidt and Trout, 1982).

3.4 Evaluation of Processes

The evaluation was divided into two main processes, cold boning and hot boning. Electrical stimulation and freezing were considered as additional processes to both CB and HB.

3.4.1 Cold Boning or Conventional Process

The results for all the different parameters under study obtained from CB, CBF and CBES processed meats can be seen in Tables 15, 16 and 17. The measurement of WHC for CB, CBF and CBES shows that ES and freezing produce a decline in this meat characteristic. This decrease could be due to severe protein denaturation induced in the muscle by ES and freezing. In addition to this denaturative effect, freezing is detrimental due to extended physical and structural damage in the muscle fibers.

The water binding capacity of CB meat is higher compared to CBES and CBF meats. The substantial decrease in WBC for CBES meat may be an indication that ES is affecting the protein solubility, or the unfolding of protein in an ionic environment.

That ES may decrease or negatively affect protein solubility is confirmed by the values of EC. This may be due to the formation of a more stable actomyosin complex that makes protein solubilization more

Table 15: Functional determinations in CB meat.

PARAMETER MEASURED	POSTMORTEM TIME (h) 24	72
RAW MEAT		
% WHC	78.6	78.2
% WBC	69.7	68.1
EC *	0.84	0.92
COOKED EMULSION		
% WHC	61.0	62.7
BS (arb. units)	35.0	35.3

* (ml oil/mg total proteins)

Table 16: Functional determinations in CBF meat.

PARAMETER MEASURED	POSTMORTEM TIME (h) 24	72
RAW MEAT		
% WHC	61.0	57.2
% WBC	59.0	56.4
EC *	0.72	0.84
COOKED EMULSION		
% WHC	57.2	57.2
BS (arb. units)	28.6	29.9

* (ml oil/mg total proteins)

Table 17: Functional determinations in CBES meat.

PARAMETER MEASURED	POSTMORTEM TIME (h) 24	48	72
RAW MEAT			
% WHC	77.1	74.2	73.4
% WBC	50.0	48.2	52.1
EC *	0.61	0.72	0.97
COOKED EMULSION			
% WHC	56.1	59.3	59.0
BS (arb. units)	35.75	35.38	36.63

* (ml of oil/mg of total proteins)

difficult. However, as postmortem time increases the EC values increase for CBES . At 72 h postmortem CBES has a higher EC than CBF, which may indicate that the limited proteolysis during aging enhances the proteins'ability to form emulsions.

After the sausages were cooked, CB emulsions showed the highest values for WHC and BS. However, emulsions prepared with CBES meat had values close to CB sausages, indicating that after thermal processing CBES meat proteins may have reacquired aspects of their functionality. As was discussed previously, integrity of myosin molecules is an important factor in the development of the protein matrix and the subsequent gel strength.

3.4.2 Hot Boning

The hot deboning process was tested with electrically stimulated and unstimulated samples, the results obtained for the different determinations can be seen in Tables 18 and 19. With respect to WHC, HB meat showed lower values than HBES meat. This may indicate that the loosening of the protein structure caused by ES allows protein to bind more water. However, the more extended shortening in HB muscle may diminish the available spaces in the myofibrillar structure to bind water. Loosening and shortening of muscle fibers both occur in meat that has been electrically stimulated and hot boned, hence both actions may stress and weaken the protein structure. This effect may account for the decrease of WBC and the increase of EC.

The integrity of the muscle proteins is more affected in HB than in CB processes. This is perhaps related to increased proteolysis. Hence,

Table 18: Functional determinations in HB meat.

PARAMETER MEASURED	POSTMORTEM TIME (h) 24	48	72
RAW MEAT			
% WHC	74.3	71.5	71.0
% WBC	71.3	57.4	58.6
EC *	0.87	1.03	1.11
COOKED EMULSION			
% WHC	56.7	57.9	52.0
BS (arb. units)	30.4	30.4	30.1

* (ml oil/mg total proteins)

Table 19: Functional determinations in HBES meat.

PARAMETER MEASURED	POSTMORTEM TIME (h) 24	48	72
RAW MEAT			
% WHC	80.3	76.8	75.5
% WBC	71.4	65.1	52.9
EC *	1.09	1.20	1.25
COOKED EMULSION			
% WHC	53.9	57.4	55.0
BS (arb. units)	30.1	30.1	31.8

* (ml oil/mg total proteins)

the presence of smaller segments of protein may produce a thinner interphase layer over the fat droplet. Subsequently, the larger numbers of molecular segments per unit weight of protein might give a larger EC but a weaker gel strength. This probable mechanism is supported by the results of WBC and EC.

Furthermore, the data obtained for WHC and BS in the cooked emulsion seems to confirm the mechanism suggested above, although ES does not seem to change binding ability of the proteins after the meat has been emulsified and thermally set.

4. CONCLUSIONS

The water holding capacity of meat is affected by hot deboning, electrical stimulation and freezing. Electrical stimulation improves WHC in HB meat, probably due to the reduction of the disruption of muscle fibers caused by cold shortening. Electrical stimulation induces a decrease of WHC in CB muscle, possibly as a consequence of the loosening of cell structure caused by the electrically induced contractions. Freezing has been shown to have the most negative effect on WHC . This could be attributed to protein denaturation and to the damage caused to the sarcolemma by ice crystals.

Electrical stimulation and freezing demonstrated a negative effect on WBC. However, the results obtained were insufficient to draw any clear conclusions as to why this should occur.

The results for EC demonstrated that hot boning either electrically stimulated or unstimulated meat increases the ability of protein to emulsify oil. The process probably causes more extensive protein hydrolysis by endogeneus enzymes. Electrical stimulation of CB meat was found to decrease EC as much as freezing, although the decreased EC in frozen and CBES meats may be attributed to different reasons.

The strength of the protein matrix depends on the functionality of the protein. Water holding capacity and BS are measuring matrix strength in different ways. Both determinations are mainly affected by the time of muscle excision in the unfrozen samples. Electrical stimulation does not seem to alter protein functionality after proteins have been emulsified and thermally denatured by cooking.

This work was mainly oriented to investigate the effects of HB, ES and freezing on the functional properties of the final product. In the light of these results the main conclusions established are that any one of the processes studied is less severe than freezing with respect to protein functionality and a high value of EC does not necessarily mean a good binding strength in the final product.

The results of this study reveal that neither ES nor HB nor combinations of these processes can substantially improve the functional properties of meats used in sausage making. As the use of frozen meat in the processed meat industry has been widely accepted, the incorporation of HB and ES in the packing plant will probably not affect processing quality significantly, since neither decreases the protein functionality as much as freezing.

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